

What weapons for what war?

The US Department of Defense is being given a predictably but understandably rough ride by the US Congress. It should prepare itself for the end of the Strategic Defense Initiative and should put the B2 bomber on ice.

THE usual case for building new weapons systems is that they may be needed in some future war, but that seems not strictly to apply to the B2 bomber over which the US Congress has been agonizing in the past few weeks. The aircraft is a remarkable exercise in high technology. Its chief characteristic, according to the US Air Force, is that it has a skin that makes it virtually undetectable by ground-based radar (presumably because the skin is made of organic materials that absorb rather than reflect at military radar frequencies), but it also has enormous range and carrying capacity — roughly a score of nuclear bombs. The cost of development so far is believed to exceed \$22,000 million. The need now is to decide whether to commit a further \$4,000 million for building the first of a planned fleet of 132 copies of the machine, the total cost of which will not be far short of \$50,000 million.

Understandably, given continuing anxiety about the US budget deficit, the high cost of the project seems at the front of most people's minds. Does it make sense to pay more than \$400 million for a single aircraft? The simple answer is that there is no absolute rule for telling what should be the cost of a military machine, but that, when large civil aircraft may sell on commercial markets for up to \$200 million, it is not entirely unreasonable that military aircraft, packed with handmade electronics, cost so much. The more serious issues are whether the procurement of the B2 is the most effective contribution to the security of the United States that might be secured for \$50,000 million and, whether, even if it were, the United States can afford such spending. The short answer is that the spending should be postponed until another year.

The fate of the B2 bomber will depend on the outcome of the bilateral START negotiations on strategic weapons just resumed at Geneva. There seems a good chance that the United States and the Soviet Union will reach an agreement that the numbers of strategic warheads deployed against each other should be reduced by roughly a half. The United States is working on the assumption that US nuclear forces will depend on the now-classic triad of land-based and submarine missiles and airborne warheads carried by bombers. But is that necessarily the case? In the past few weeks, the US Air Force has been giving members of the Congress unconvincing tales of how B2 bombers could be used in the wake of a substantial nuclear exchange to ensure that surviving hostile command posts are destroyed, which seems to have more

bearing on the prosecution of nuclear war than on its deterrence.

If the outcome of the START negotiations is that the two major nuclear powers are restricted to roughly 2,500 nuclear warheads each, there will be a strong case for believing that the United States would be better placed with its eggs in two baskets only — submarine and land-based missiles. And the view of the US Air Force that Congress must buy at least a few examples of the aircraft so as to provide a bargaining counter at Geneva makes no sense. The \$22,000 million spent on development so far is a sufficient guarantor that the United States is serious about the B2. That is why the Congress should settle at this stage only for continued development, not procurement.

The Armed Services Committee of the House of Representatives has already voted in favour of reducing the cost of the Strategic Defense Initiative (SDI) for the financial year ahead to \$1,600 million — less than half what the administration had asked for. If that is the final outcome, it will imply that SDI lapses into a development programme, which is what it should have been all along. There seems no doubt that the START negotiations will not succeed without an undertaking that the Anti-Ballistic Missile Treaty is strictly interpreted, which would prevent the deployment of space-based weapons. Nobody will complain if the Pentagon thereafter continues to explore the potential of some of the schemes that were the objectives of SDI, but there is no case for doing more than that. And that conclusion should not be a matter for regret, but for rejoicing: like the Soviet Union, what the United States most stands to gain from arms control is relief from a haunting budget crisis. □

Using fetal tissue

The British government has been given good advice on the conditions under which fetal tissue may be used.

LAST year's great fuss about the experimental use at a Birmingham hospital of transplanted fetal tissue for the treatment of parkinsonism should have been neatly laid to rest by the report of a committee under Dr John Polkinghorne, which appeared last week. The committee puts forward rules for the use of fetal material of all kinds that, while more restrictive than those now in force in



Britain, are clear, workable and an intelligent way of accommodating the interests of those who believe abortion to be an abomination within the framework of what might well become a common medical procedure. But the Polkinghorne proposals will raise problems for the British government, which has not responded with legislation in more than two years to the report of the Warnock Committee on the use of embryonic material.

The basis of the new proposals is that a living fetus (but not the associated placental material) should command the respect to which all living human beings are entitled, and that fetuses that have died should be dealt with as carefully as are cadavers. What this implies is that material from living fetuses shall not be used for research or therapeutic procedures, that there must be informed consent (primarily by the mother), that there should be a strict separation between the intended use of the fetal material and the means by which it is acquired (implying that abortions should not be timed to suit the needs of intended users and that no money should change hands) and that the use made of fetal material should be regulated by ethical committees, constituted so as not to represent only the interests of the putative users. The Polkinghorne committee rejects the argument that artificial abortion, even on therapeutic grounds, is an immoral practice so fetal material derived from such procedures is morally tainted.

These guidelines differ from those in force in Britain since 1972 in their insistence on the informed consent of the mother of a fetus and on the strict separation of the interests of the sources and users of fetal material. But these requirements are reasonable, and are entirely consonant with modern practice in other fields, genetic manipulation for example. The proposal that the transfer of fetal material should be undertaken by an intermediate authority, perhaps an elaboration of the Medical Research Council's tissue bank, is similarly the most effective way of making sure that money does not change hands improperly. Neither researchers nor clinicians have grounds for complaint at what is proposed.

The logical difficulty, for the British government, is that the Polkinghorne proposals are less stringent than those suggested for the use of human embryos in research. In particular, the Warnock committee recommended an absolute interdiction of the use of embryos more than 14 days old that would be enforced by new legislation. But it is hard to understand why the regime now proposed for the use of fetal material would not also suffice to meet the Warnock committee's proper demand that human embryos should be accorded the respect due to all living human beings. The British government, which has a commendable record of initiative in tackling the problems of how to use new techniques in biology, might usefully offer Polkinghorne as an answer to Warnock — and devote the energy liberated to securing an international convention to make the new guidelines generally applicable. In this field, as with genetic manipulation, there is too great a danger that national arrangements will be subverted by taking advantage of laxer rules elsewhere. □

British musical chairs

Britain's new minister in charge of research and higher education has some formidable tasks ahead of him.

THE British convention that ministers in charge of government departments are chosen for their versatility, not special knowledge, is nicely illustrated by last week's round of musical chairs decreed by Mrs Margaret Thatcher, the prime minister. Most attention has been paid to the demotion of Sir Geoffrey Howe, the experienced Foreign Secretary, whose continuing function in the British government will be that of Leader of the House of Commons, but the same reshuffle has translated Mr John MacGregor, last week's Secretary of State for Agriculture, into the shoes of Mr Kenneth Baker, last week's Secretary of State for Education and Science. By the standards set by his predecessor, Baker (who becomes chairman of the Conservative Party) was a success, but there is no reason why MacGregor, who is a brisk and intelligent man, should not be as successful. It is bad luck that his tenure at the agriculture ministry should have coincided with the emergence of justifiable public anxiety about the safety of the food available in British shops.

But MacGregor will find some awkward problems on his new desk, not the least of which is that provided by the Advisory Board for the Research Councils (ABRC), which is again asking that more money should be spent on the support of British civil science (see page 333). He should not be surprised that, a year after his predecessor added roughly £100 million a year to the science budget for each of the next three years, he is being asked to do much the same again. The truth is that the funds now asked for are required to make good the deprivations of the past decade as well as to pay for basic research squeezed out by the extra demands made of the British research councils during the same period. But the new minister should acknowledge that his response may properly await ABRC's decision about the future organization of the research councils (see *Nature* 339, 645; 29 June 1989) and that there are some features of the British research enterprise that cannot be put right simply by the provision of extra funds.

The most ominous feature of ABRC's advice is the declaration that British research is now hampered by the scarcity of skilled people. That is no surprise, after a decade in which young people have been discouraged from regarding the prosecution of research as a rewarding career. ABRC may also have noted that matters can only worsen as greater proportions of the surviving research profession in Britain realize that they can do better for themselves by moving elsewhere in Europe. More money is a necessary but not a sufficient condition for the reinvigoration of British science. MacGregor will also have to find a way of helping the British research profession win back its self-respect. He could do worse than begin with a few well-sounding speeches. □

Strasbourg gets the vote for 'Frontiers' HQ

- Japanese government investing in Europe
- Britain and Italy among also-rans

Tokyo

AFTER years of deliberation, Japan, in consultation with representatives of the Western summit nations (United States, Britain, France, Italy, Canada and West Germany) and the European Communities, has decided to locate the office for the Human Frontiers Science Program in Strasbourg, France. The highly political decision, which runs counter to the recommendations of the Japanese government's own academic advisers, was strongly influenced by an offer from both France and the local government of the Strasbourg area to provide funds for Frontiers — the first financial contribution from a foreign nation to the Japanese-led programme.

Frontiers was first announced by Japan's Ministry of International Trade and Industry (MITI) in 1986 as a major thousand-million-dollar programme of international bioscience research intended to repay Japan's 'debt' to the basic research of Western nations. But the programme was eventually much reduced in size and now stands at only about \$20 million for fiscal year 1989, its first year of full-scale operation. Nevertheless, the programme is unique in that the Japanese government is for the first time putting its taxpayers' money into support of basic research overseas.

Frontiers will be run as a non-profit foundation that will award grants and postdoctoral fellowships for research on the brain and molecular recognition to international teams of scientists from the summit nations. The foundation will also support international workshops.

At first, the Japanese government planned to locate the office in Switzerland (see *Nature* 334, 281; 1988) but the summit nations objected. London was recommended by Japanese academic advisers because many academics speak English and because Britain has a long tradition of scientific research. British government officials offered a Medical Research Council building as a possible site. But the French government objected and proposed an alternative site in Strasbourg. Rome was nominated by the Italian government.

At an intergovernmental meeting in Berlin last Friday, it was finally agreed to choose Strasbourg. According to a spokesman for Frontiers at the Science and Technology Agency (STA), Strasbourg was chosen because the local government and people of Strasbourg were "very enthusiastic" to invite Frontiers and

offered "beneficial" conditions, including financial support.

Over the next three years, the local and French national governments will provide about ¥500 million (\$3.5 million) to cover the costs of rent (for the foundation's office), 15 postdoctoral fellowships and one or two international workshops. This represents only a few per cent of the amount that STA and MITI hope the Japanese government will invest in Frontiers over the next three years, but France's contribution will help the two government agencies in their negotiations with the Ministry of Finance. The French decision is also expected to trigger contributions from other nations.

This month, Japan will send out formal requests to all summit nations for financial support. According to the STA spokesman, West Germany has indicated that it will support a number of fellowships and it is hoped that other nations will follow suit. A number of non-summit nations, including Sweden, Switzerland, Australia, the Netherlands and Finland, have also shown interest in joining the programme. A decision on their participation will be made by the board of trustees and the science council of Frontiers, members of which are soon to be nominated by the summit nations. STA's two main criteria for selection of non-summit countries will be their ability to contribute scientifically and financially to the programme.

The Japanese government will begin soliciting applications for this year's grants, fellowships and workshops in the middle of this month under the temporary administration of the Japan Science Foundation, an organization in Tokyo run by STA and MITI. The unopened applications will then be passed to the foundation in Strasbourg when it opens in October.

David Swinbanks

COMPUTER SECURITY

Hacker's intentions key to court case

Washington

NINE months after the event, criminal charges are to be brought against Robert T. Morris Jr, the architect of the computer virus that disabled thousands of computer installations linked by the Internet network (*Nature* 336, 97; 1988). Morris, a graduate student at Cornell University, was due to be arraigned under the 1986 Computer Fraud and Abuse act on 2 August in Syracuse, New York, on charges of "unauthorized access" to a number of computers at federal facilities.

Morris' identity as the perpetrator of the virus programme has been clear from the outset, but he has maintained in public that he intended to only demonstrate the ease with which security routines on Internet could be by-passed, and that the wildfire spread of the virus across the country was unplanned. For a successful prosecution under the terms of the 1986 Act it would be necessary to prove that the unauthorized access was "intentional", not merely that it occurred. Although a number of cases have been brought under the Act, the majority

Robert Morris, pictured in 1988 on graduation from Cornell, claims that his computer 'virus' exposed the weaknesses in Internet's security measures and that the effects have been beneficial.

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

are still pending, and there is no precedent for this kind of prosecution.

That a criminal charge has finally been made despite the uncertainty of success is taken to indicate an official desire to make an example of Morris. His supporters, on the other hand, claim that the incident was a valuable demonstration of the looseness of the system, and that it has galvanized computer scientists and Congress into remedial action (*Nature* 340, 252; 1989).

David Lindley

Phobos probes provide data for some



MORE new photographs from the ill-fated Soviet Phobos missions were made available last week — this one showing the moon Phobos rising above the curvature of Mars. Although both Soviet probes malfunctioned before completing their missions, most of the plasma data had already been gathered. The planetary surface and atmosphere experiments were the most badly affected, accumulating only some 10 per cent of the data hoped for. *Nature* is to carry a special supplement later in the year with the first detailed results from the Phobos missions. □

Congress awards few stars

Washington

THE annual political showdown between the US Congress and the administration over funds for the Strategic Defense Initiative (SDI) is likely to leave the Department of Defense (DoD) with a little less to spend on the programme next year than the \$4,000 million it spent this year. President George Bush inflicted the first wound to SDI when he "reluctantly" sliced \$1,000 million from the DoD's request of \$5.6 million next year, in a round of across-the-board spending cuts. Not unpredictably, when it was the turn of the House of Representatives last week, the SDI programme emerged decidedly leaner at \$3,100 million. This week it is in the hands of the Senate where SDI has more friends than in the House; the Senate Armed Services Committee has recommended that a budget of \$4,600 million be approved. After that, if the

research on newer concepts, especially the 'brilliant pebble' interceptor rockets, would be cut. These may be exactly the effects that Congress hopes for in its wish to maintain a research programme but postpone deployment. Bush, who is "personally and deeply committed" to SDI, said he was disappointed at last week's cuts and emphasized the importance of SDI as a bargaining chip in arms negotiations. Few now consider former President Reagan's vision of a comprehensive defence shield to be realistic.

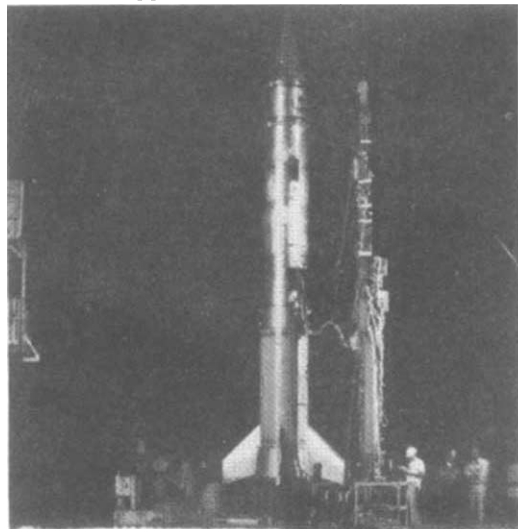
Congress has cut the SDI budget request every year but one since the programme began in 1984. But opposition is stiffening as deployment approaches. The cuts were approved this year by a higher majority than in previous years, although no restrictions were placed on specific programmes. And the House of Representatives reaffirmed its opposition deployment last week by passing unanimously an amendment urging Bush to seek the dismantling of an existing Soviet antisatellite weapon (ASAT), to negotiate for the "strictest possible limitations" on ASATs. It also called for a comprehensive report on US antisatellite weapon programmes including an assessment of the antisatellite potential of SDI technology. A strict ASAT limitation treaty would restrict deployment of SDI systems as well as blocking several ASAT programmes.

The amendment also called for a report on the interference of orbiting nuclear reactors on scientific satellites. This is seen as another backdoor route to blocking SDI deployment as nuclear reactors in space will be essential to provide power for space-based weapons such as charged particle beams and free electron lasers. In the House of Representatives, legislation is pending that would call for an international treaty to prohibit the use of nuclear power in orbit, except in deep space missions.

Since 1984, \$17,000 million has been spent on SDI research and the Pentagon wants to double that over the next five years. An estimated \$80,000 million will then be needed for deployment. These requests are unrealistically high, and future tight budgets are likely to restrict the research and testing on which to base decision-making, pushing back the deadline for a decision on deployment.

Critics of SDI are likely to focus opposition on a series of major field tests planned to take place over the next two years.

Some of the trials may be argued to violate the Anti-Ballistic Missile Treaty.



SDI in practice: Above, the BEAR (Beam Experiment Aboard Rocket) payload was launched by this Aries rocket in July, the first test of a neutral particle beam in space. Right, the brilliant pebbles interceptor is still at the model stage at Lawrence Livermore. The scale is a one-foot rule.



ritual of previous years is repeated, the House and Senate will compromise by splitting the difference.

The Strategic Defense Initiative Organisation (SDIO) says that with less than \$4,000 million the longer-term technologies will not be available in time to offset Soviet countermeasures to the first phase of the strategic defence system. It also says that a decision on deployment would be delayed by two years, the workforce would be cut by more than 6,000, and

Star wars in 1989

Boost phase defence

■ **Surveillance and Tracking System:** Satellites equipped with a telescope and sensor that detect and track ballistic missiles will be the first SDI systems deployed.

■ **Space-based interceptors:** Testing has begun of guided interceptor rockets that will destroy missiles as they rise through the atmosphere by colliding with them at high velocity. The final plan calls for 300 orbiting 'garages' each containing multiple rockets.

■ **Brilliant pebbles:** Thousands of three-foot long interceptor rockets each with a sensor and computer are planned to orbit the Earth in swarms. In the event of an attack, each would independently seek out incoming missiles and destroy them by impact.

■ **Chemical space-based laser:** Orbiting platforms could direct extremely powerful infrared laser beams onto missiles. The 'Zenith Star' space experiment with the Alpha chemical laser is scheduled for the mid-1990s.

Boost — Midcourse defence

■ **Neutral particle beams:** Warheads are destroyed with beams of high-energy atomic particles. The first test in space was carried out this year but a practical weapon could not be ready before the end of the century.

■ **Space-based electromagnetic rail gun:** rail guns could destroy weapons with 2 kilogram projectiles fired at 2–3 kilometres per second. To destroy missiles in boost phase would require speeds of 12 kilometres per second. Ground tests have reached projectile velocities of 2 kilometres per second with 150-gram projectiles, making full-scale development unlikely before the late 1990s.

■ **Free electron laser:** The free-electron laser, which would destroy warheads with a beam of high-energy electrons, is the most promising of the ground-based beam weapons. There are two competing types; this year SDIO will choose between them and award a contract for construction.

Midcourse — Terminal defence

■ **The Airborne Optical Adjunct:** Modified Boeing 767 aircraft will be equipped with long-wave infrared and optical sensors. About 40 would cover the United States and detect incoming warheads and separate out decoys. The first test flight is planned for 1990.

■ **Exo-atmospheric and endo-atmospheric reentry interceptor subsystem:** Six-foot long interceptors launched from the ground would destroy weapons by impact before or after they enter the atmosphere. The first of a series of tests is due this year. □

They include further tests in space of neutral particle beam lasers, the first flight test of an endo-atmospheric interceptor, the first flight of the airborne optical adjunct and the first major tests of the effects of the atmosphere on the free-electron laser.

Christine McGourty

DRUGS TESTING

Parallel trial controversy

Washington

THROUGH a premature announcement of a new "parallel track" for the testing of AIDS drugs, Anthony Fauci, director of the US National Institute of Allergy and Infectious Diseases, has set off a chain reaction over how US drug approval procedures should be changed in the light of the AIDS pandemic. Fauci's announcement just over a month ago that the government may soon sanction the distribution of unapproved drugs to people with AIDS outside controlled drug trials triggered media reports and a congressional hearing. Congressional interest, in turn, has prompted James O. Mason, Assistant Secretary of Health, to instruct Food and Drug Administration (FDA) commissioner Frank Young to find out just how such a scheme would work.

The deluge of criticism from AIDS activists over the speed of drug testing by FDA has already brought about major changes in the drug-approval process in the past three years. The first new measure was the 'treatment IND' (investigational new drug) under which companies could sell drugs for serious and life-threatening diseases after the first two phases of tests — for safety and efficacy — are complete, but before the final phase of testing on a larger number of patients begins. Next came the '1AA' designation to streamline the FDA's processing of the paperwork for AIDS drugs. And last year,

the FDA proposed dropping the expanded Phase III trials altogether for drugs for life-threatening diseases, in favour of a 'Phase IV' surveillance to study side-effects once the drug is on the market. The latest 'parallel-track' proposal would allow people with AIDS to receive 'safe and promising' drugs even earlier — after Phase I safety tests.

Young says he and Fauci have been discussing ways to make promising AIDS drugs more available for nearly two years, but that Fauci's public announcement came as a "surprise". He does not think a 'parallel track' would decrease the number of participants in controlled clinical trials: people get free treatment — not just the drug — in a clinical trial, and the 'parallel track' could be structured so that it opens up only once established clinical trials are filled. But Young would prefer to see the treatment IND improved instead.

Drugs companies are not expected to embrace another proposal for them to give drugs away free. A statement issued by the Pharmaceutical Manufacturers Association at the congressional hearing two weeks ago was ambivalent. But Bristol-Myers — the maker of dideoxyinosine (ddI), an AIDS drug less toxic than AZT — may lead the way. The company has announced it will give ddI to people with AIDS outside its Phase II clinical trials, set to begin in September, if the FDA permits.

Carol Ezzell

AIDS SURVEY

No sex please, we're American

Washington

WITH the blunt words "The Public Health Service is directed not to proceed with the study", the US House of Representatives Appropriations Committee last week scotched an \$11 million budget item that would have provided funds for a survey of sexual behaviour in the United States. A \$2-million pilot survey, for which funding has already been obtained, is awaiting approval by Louis Sullivan, Secretary of Health and Human Services (see *Nature* 340, 90; 1989).

Howard Silver, executive director of the lobbying group COSSA — the Consortium of Social Science Associations — suggests that Congress's peremptory dismissal of the survey was catalysed by recent agitation over the "Mapplethorpe affair". Washington's Corcoran Gallery cancelled a planned exhibit of works by the photographer Robert Mapplethorpe, who died of AIDS earlier this year, out of concern that the arguably "pornographic" nature of some of the photographs would jeopardize the Corcoran's support by the National Endowment for the Arts (NEA). The Mapplethorpe exhibit was put on privately instead.

With sex such a sensitive topic in Washington, the House decided that a survey of sexual behaviour was not "an appropriate use of public funds". Sullivan could now, according to Silver, use the cancellation of the full survey as a reason to abandon the pilot survey too, and avoid any further controversy. On the other hand, if the pilot survey were to proceed, and produce valuable insights into the future spread of AIDS, researchers would have ammunition with which to return to Congress and ask for the \$11 million again.

David Lindley

NUCLEAR ENERGY

Waste exports opposed

Munich

THE trend toward European cooperation in nuclear energy continued last week when West Germany signed an agreement to ship nuclear fuel to Britain for reprocessing. British Nuclear Fuels PLC (BNFL) agreed to reprocess 6,000 tonnes of fuel at its new Thorp plant beginning in 1999 for an estimated cost of DM5,000 million (about £1,600 million).

But the pro-nuclear West German government will not be able to export its political problems with nuclear power so easily. Opposition Social Democrats (SPD), who are in power in six of eleven *Länder* (states), have been threatening to bring the centre-right government before the federal constitution Court over the right to reprocess nuclear fuel outside West Germany.

The issue is safety. The West German Atomic Law requires the government to guarantee that used fuel elements will be disposed of in an environmentally safe manner; but it makes no prescription about whether or where the fuel should be reprocessed. Once the fuel leaves West

Germany, the opposition claims, the government can no longer guarantee its safety. SPD politicians would like reprocessing to stop and the fuel to be brought directly to nuclear waste dumps.

Environment Minister Günther Jansen (SPD) of Schleswig-Holstein is prepared to shut down the three nuclear reactors in that *Land* if the government does not abandon reprocessing entirely.

West Germany signed a similar agreement with the French company Cogema in April to reprocess fuel at La Hague in northwestern France (see *Nature* 338, 611; 1989). The agreements allow West German utilities to reprocess fuel at a much lower cost than would have been possible at the abandoned Wackersdorf plant.

The spokeswoman for the Federal Ministry of Environment and Reactor Safety, Marlene Mühe, said the government may try to force Schleswig-Holstein to accept the government view, which could lead to a court battle. SPD spokesman Michael Weidemann said Schleswig-Holstein would prefer further negotiations.

Steven Dickman

AIDS RESEARCH

French group moves on two fronts

Paris

THE French national association for AIDS research (ANRS), which was officially inaugurated last month, has chosen its first two 'co-ordinated actions'. The first is an epidemiological study of the prevalence and incidence of infection by the human immunodeficiency virus (HIV) in France. The study, to be directed by Dr Jean-Baptiste Brunet, will report annually on the epidemic.

The second programme will work on anti-HIV vaccines. It is to be directed by Professor Marc Girard, scientific director of Pasteur-Vaccins. The programme will coordinate the work of laboratories currently working on candidate vaccines and will tie them in with the two major French vaccine producers, Pasteur-Vaccins and Transgene.

Peter Coles

Bills to counter terrorists

Washington

LEGISLATION that would make it a crime in the United States to make or stockpile biological agents that could be used as "weapons of mass destruction" seems set to be passed by Congress.

Bills in both the Senate and House of Representatives have attracted many sponsors and are expected to be voted on this year.

The legislation is intended to implement the 1972 Biological Weapons Convention, the treaty banning the development of biological weapons signed by 103 nations including the Soviet Union. The United States ratified the treaty, but the lack of political support for arms control in the late 1970s and early 1980s made it difficult for Congress to pass criminal legislation outlawing biological weapons. Current concerns over possible terrorist activities have now convinced Congress that such laws are needed.

The Bush Administration also supports the idea. Ambassador H. Allan Holmes, now Assistant Secretary of State, said at a congressional hearing last week that there is no evidence that any specific group or country is developing biological weapons, but that approximately ten countries have the capability to do so.

Ambassador James F. Leonard, one of the negotiators of the bioweapons treaty, says the law will create "confidence

abroad" that the United States will follow up on treaty obligations — particularly important now because of current negotiations for a treaty banning chemical weapons.

Moves are also afoot to prove to other countries that the United States is not making biological weapons. Representative Wayne Owens (Democrat, Utah) last week tacked an amendment onto next year's defence spending bill requiring the military to publish annually a list of the biological agents it is using to develop defensive measures — allowed by the biowarfare treaty — in the event of an attack with biological weapons. A bill has also been introduced to transfer all bio-defence research to the National Institutes of Health.

Both the current House and Senate bills specifically exempt research on biological agents "for prophylactic, protective, or other peaceful purposes". But they differ over whether the government must be able to prove malicious intent, or whether government officials will be able to seize and destroy suspect cultures, and ask questions later, leaving it up to an individual to prove they were performing legitimate research. Which side will carry the "burden of proof" will be decided in the next few weeks, with advice from the relevant federal agencies.

Carol Ezzell

GENETIC MANIPULATION

Ambitious plans for Australian gene-shearers

Sydney

IN keeping with its commitment to making science pay, Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) has announced a joint venture which may be valued at A\$67.5-million, the largest ever undertaken by the organization. The venture, with the French seed company Groupe Limagrain and an Australian company yet to be named, will try to find commercial applications for the 'gene-shears' technology developed by Jim Peacock, head of the CSIRO Department of Plant Industry.

CSIRO holds the world patents to the technology, which exploits the natural ability of some RNA molecules to undergo self-catalysed cleavage to design RNA enzymes that would cleave the RNA transcripts from specific genes. The aim is genetically to engineer organisms so that they produce an RNA enzyme that will switch off a specific gene, as desired.

According to Peacock, possible commercial applications for gene-shears technology are very wide, including the control of human genetic and viral diseases, the ability to switch off genes that make seeds

or tissues inedible or unpalatable, the production of new hybrid plant varieties and the control of contamination of the bacterial cultures used in the brewing and cheese-making industries.

"This technology has a far greater potential than CSIRO could look after itself. Depending on whether we can demonstrate that gene-shears works in whole plants and animals then this joint partnership will enable CSIRO to target suitable commercial ventures", Peacock said.

According to a CSIRO spokesperson, much of the investment by the French and Australian partners will go to Australian universities. But Peacock maintains that, if Australian universities do not measure up, the money for research will go overseas. "Wherever possible contracts will go to Australian groups. This is one of the few occasions in Australia when there is a substantial amount of money going into one type of technology. We will do our utmost to spread that influence in the Australian research industry. But if the best commercial groups are overseas that is where we will go. There will be no false nationalism."

Tania Ewing

US — Soviet journal launch

San Francisco

AFTER nearly seven years of planning and bureaucratic negotiations, a quarterly journal produced jointly by Soviet and US scientists is due out this month.

The main aim of *Science & Global Security* is, according to editor Harold Feiveson, a senior research scientist at Princeton University, "to help foster, among scientists and other analysts, an open discussion on problems of arms control and global environmental issues".

The inaugural issue focuses on "space reactor arms control", and addresses such topics as infrared monitoring of nuclear power in space and the environmental hazards of space-based nuclear power.

The idea for the journal originated in 1983 with Evgenii Velikov, vice-president of the Soviet Academy of Sciences and former chairman of the Committee of Soviet Scientists for Peace and against the Nuclear Threat. Velikov sought out Princeton's Frank von Hippel as a US ally — with the goal of producing a technically strong, non-governmental publication that could be used as an archival base for future studies. At first, the idea was to focus on arms control and military issues, but the scope quickly broadened to include space and the environment.

The idea was developed further in the United States and the Soviet Union for at least two years, and was then delayed when the Soviets initially refused approval, says Feiveson. Velikov remains on the board of editors. His successor at the Committee of Soviet Scientists, Roald Sagdeev, joins von Hippel as co-chair of the 14-member editing board, which includes five Soviets and nine Americans.

It is too early to tell how much support the new journal is gathering. Joel Primack, a physics professor at the University of California, Santa Cruz, and the main author of the overview article on space reactors in the first issue, says the quarterly journal fills a vital niche. "There are a number of magazines for non-technical articles", he says, "but until this one was started I do not believe that there has been a technical journal where you can publish articles with equations and detailed analyses and footnotes and so forth."

Initial attempts to find a Soviet publisher have become bogged down, perhaps because of traditional wariness of 'political' issues, so a Soviet publisher is still being sought. But the journal will be published in English by Gordon and Breach Science Publishers.

Individual subscriptions, available through Gordon and Breach in either London or New York, cost \$34 or £24 a year.

Robert Buderl

NUCLEAR ACCIDENTS

Medvedev testifies

London

DR Zhores Medvedev, the biologist and former dissident who was deprived of his Soviet citizenship under Brezhnev, returned to the Soviet Union last week to testify before a special commission of the Supreme Soviet on Nuclear Safety. Medvedev is the author of *Nuclear Catastrophe in the Urals*, the only book so far on the accident in 1957 which until a few weeks ago had been covered by a veil of official secrecy. The hearing was chaired by Yury Shcherbak, author of a major work on the Chernobyl disaster (*Chernobyl: A documentary Story*, Macmillan, 1989) and now chairman of the Supreme Soviet's subcommittee on nuclear ecology.

Speakers at the hearing included Boris Nikipolov, first deputy minister of medium machine building (the cover-name for the nuclear industry), who testified that the accident, on 29 September 1957, had taken place at a "defence enterprise in the southern Urals", where there had been "violations" in the cooling system of a 300 cubic metre concrete vessel, used for storing radioactive waste. There was a partial evaporation of water, Nikipolov said, leading to a rise in temperature and the consequent explosion of acetate-nitrate salts. The seal on the vessel was ruptured, and there was an emission of radioactive material into the environment. "Twenty million curies of radioactivity" were ejected from the vessel, Nikipolov said, but 90 per cent of this fell in the immediate vicinity of the vessel. The remaining 10 per cent was eventually deposited over some 15,000 square kilometres of the Chelyabinsk, Sverdlovsk, and Tyumen regions, from which some 270,000 people had to be evacuated and 100,000 hectares of land taken out of agricultural use.

Medvedev's evidence related mainly to the medical aspects of the disaster, which took place, he stressed, at a time when the hazards of exposure to ionizing radiation were not fully appreciated, and to his own role in revealing the catastrophe to the West. He related how his first mention of the disaster — in a general survey of Soviet science — had attracted a flood of criticism from Western supporters of nuclear power, who had accused him of being a KGB agent, sent to discredit the US nuclear programme.

Reviewing the session, Shcherbak observed that this was the first step in a "major, very complicated and very long march" towards openness in matters relating to nuclear power.

While Zhores Medvedev was testifying before the commission on nuclear safety, his dissident twin brother Roy was working as head of another special commission, investigating the activities of T. Gdlyan,

an official attached to the Soviet chief prosecutor's office. Gdlyan had been responsible in 1983 for the prosecution of Johannes Hint, an Estonian physicist and Lenin Prize winner for "embezzling state property on an especially large scale", (see *Nature* 354, 307; 1983). Hint died in prison in 1985, but was posthumously rehabilitated earlier this year. It then emerged that Gdlyan was still in high official favour, and was now in charge of investigations into the notorious corrup-

BRITISH RESEARCH

No way to prepare for 1992

London

AS 1992 and the European "single market" approaches, British science is falling behind, according to the Advisory Board for the Research Councils (ABRC). In a report to Kenneth Baker, the former Secretary of State for Education, published on 27 July, the ABRC points to "worrying signs the UK's share of scientific output is declining", and says that a boost of £360 million is needed over the next three years to prepare for 1992.

The government announced last autumn an increase of nearly £100 million in the science budget over the next three years but the ABRC, which advises the government on the distribution of research funds to the universities, says this represents a reduction of 3 per cent in real terms, and that even allowing for this increase the UK is spending between £150 million to £200 million per year less than its main European competitors.

Britain's ability to contribute to the international debate on environmental change is questioned. The United Kingdom is involved in a number of important international research programmes, says the report, but "our assessment is that without additional resources, UK scientists will be unable to play a full role in these."

Britain will have to be content with a reduced role in the European space programme and British scientists will miss the chance to research into the ability of the oceans to absorb the 'greenhouse' gas carbon dioxide. A number of health programmes are under threat, including plans to mount clinical trials in breast cancer screening, a vaccine for whooping cough and studies of the causes of deaths amongst the elderly in winter. And unless action is taken soon to improve the climate for science "there will be a critical shortage of suitable qualified researchers in the 1990", the ABRC says.

With 1992 on the horizon, the report also warns that Britain is poorly prepared to take advantage of the increased co-operation and exchanges of manpower

and nepotism scandals in Uzbekistan. A special commission of inquiry was hastily convened, but, in Roy Medvedev's opinion, it is liable to take some time in reaching a conclusion.

Vera Rich

■ In the article "Byelorussia collects dose" *Nature* 27 July, 1989, p.255, the fourth paragraph should begin "Unlike similar 'popular fronts' in other Soviet Republics, Adradzennie was not even allowed to hold its inaugural congress in its own republic, but had to be across the frontier, in Lithuania, while Pazniak's 'Statement to Western Journalists' had to be sent out via Latvia." □

and expertise which will emerge. Britain is "lagging well behind" France and West Germany in its support of materials science, chemistry, physics, medical and biological sciences.

The board is asking for an extra £94 million in 1990, £131 million in 1991 and £135 million in 1992. The blunt message to the government is: "Increased investment in science is essential to provide both the equipment and personnel the UK needs to meet the challenges of the 1990s".

Ben Webb

Science and Public Expenditure 1989: A report to the Secretary of State for Education and Science from the Advisory Board for the Research Councils is available from Publications Despatch Centre, DES, Honeypot Lane, Stanmore, Middx.

SPACE TELESCOPE

Demand sufficient for nine more 'Hubbles'

Washington

OUT of 556 proposals for observational programmes using the Hubble Space Telescope (HST), 162 have been selected for the first 12-month run of telescope time, due to begin about a year from now. HST should be launched in March 1990, but the first seven months is devoted to commissioning. About one-fifth of the successful proposals come from members of the European Space Agency.

The total of submitted proposals represented an oversubscription to the available observing time of almost a factor of ten, and even with the pruning that has now been done, HST time is oversubscribed about three times. High priority has been given to 108 of the proposals and the remaining 54 will be included in the schedule only if vacancies open up during the first year of operation.

Observations of particular importance are those of the cores of globular star clusters, of nearby supernova remnants, and of the central regions of active galaxies, in all of which HST's high resolution may reveal qualitatively new detail.

David Lindley

More grist for Dingell's mill

Washington

US Congressman John Dingell, who heads the powerful congressional subcommittee on oversight and investigations that pursued the Baltimore affair, is once again exercised over a case of alleged scientific misconduct. Last month, he wrote to James Wyngaarden, outgoing director of the National Institutes of Health (NIH), to ask why it was that in a recent case of alleged plagiarism, an NIH panel had "made only a finding of 'serious misconduct' but not a finding of fraud". And he asked Wyngaarden to describe "your process for criminal referrals".

Wyngaarden's reply makes it plain that NIH is not being soft-hearted. He explains that the concept of 'misconduct' includes fraud, something that is stressed in both the draft and final Department of Health and Human Services (HHS) rule concerning misconduct (see accompanying article). And he makes clear that the possibility of criminal action is being considered by the HHS Office of the Inspector General in the standard way.

But Wyngaarden makes no comment on another major part of Dingell's letter. There Dingell is critical of the role of Daniel Koshland, editor of the US journal *Science*, in the case of plagiarism.

The case (see *Nature* 340, 173; 20 July 1989) involves an allegation that a paper published in *Science* (236, 1678; 1987) by C. David Bridges, at that time a researcher at Baylor College of Medicine in Houston, plagiarized information from a manuscript by Paul S. Bernstein, Wing C. Law and Robert R. Rando of Harvard Medical School. The Rando paper had been sent to Bridges for review and was later published in *Proceedings of the National Academy of Sciences* (PNAS 84, 1849; 1987).

Dingell writes "It is disconcerting to me that in this instance a distinguished editor of a leading American journal was aware of possible scientific misconduct and not only took no steps to prevent an injustice from occurring, but actually allowed publication of the stolen material."

Dingell bases his assertion on statements in a letter that Rando wrote to Koshland shortly before the paper was published explaining there were some difficulties with it. But it is not clear that the difficulties were portrayed as serious. In his letter to Koshland, Rando explains that he had met Bridges and entered into an agreement with him that the *Science* paper should be modified to recognize Rando's work.

The relevant section of the letter from Rando says:

"I enclose an article which my co-workers and I recently published in PNAS, on very similar material.

I saw Prof. Bridges at the ARVO meeting in

Sarasota, FLA. on May 7. We reached a verbal agreement that certain changes should be made in the *Science* article to indicate that our paper had been sent to Prof. Bridges for review in July, 1986. The changes we agreed on are enclosed. I believe that it is very important that there be no discrepancies between the enclosed changes and those sent to *Science* by Prof. Bridges."

But there were discrepancies between Rando's changes and the published changes, *Science* taking the view that, as Koshland puts it, "the author is the final arbiter of changes in his own paper". The question that interests the Dingell subcommittee is whether Koshland should have read the letter from Rando as indicating plagiarism. Rando has told *Nature* that the letter "raised a little bit of a flag from me but I did not say I suspected plagiarism". But Dingell claims that Rando believes that "the letter to Dr Koshland should have raised serious concerns". Koshland's view, as reported in the latest issue of *Science*, is that Rando's letter "only raised issues about appropriate reference to his work".

The question now is whether the affair will rest as it is, or whether an attempt will be made to resolve contrary opinions by

further congressional action. Sources close to the subcommittee speak of a possible hearing on the topic of misconduct in the peer review process.

Koshland has not himself made any direct editorial statement about the affair in the pages of *Science*, instead having "turned his own reporters loose" to investigate and even to interview him. He says this is necessary because "we are very careful to keep privileged information in the editorial department, we don't leak to the news department". *Science's* investigative team have used the Freedom of Information Act to obtain documents (as has *Nature*) and not found any skeletons in *Science's* cupboards.

Bridges, an Englishman who formerly carried out research at the MRC Vision Research Unit in the Institute of Ophthalmology in London, maintains his complete innocence of any of the charges made in the NIH report. He states firmly that he has been "framed". That Bridges might be the victim of a conspiracy was discussed by the investigatory committee and Bridges points out that the preliminary NIH report stated that "vindictiveness" may have motivated actions against him. According to Wyngaarden, Bridges will have an opportunity to appeal.

Alun Anderson

New rules are finalized

Washington

STRICTER regulations to ensure that institutions have procedures in place to investigate possible misconduct in science distinguish the 'final rule' on misconduct that is about to be published by the US Department of Health and Human Services. The rule also provides a new requirement that all institutions submit "aggregate information on allegations, inquiries and investigations" each year. That information should help provide, for the first time, central records of the frequency of misconduct and allegations of misconduct.

The new rule applies to institutions receiving financial assistance from the Public Health Service and includes the National Institutes of Health (NIH).

A 'Notice of Proposed Rule Making' which set out initial proposals for public review was published last September. The final rule takes into account the 139 comments received and the two new scientific integrity offices set up this year to take care of scientific misconduct in the Public Health Service (see *Nature* 340, 3; 6 July 1989). The definition of misconduct is changed to make it clear that "honest error or honest differences in interpretations or judgements of data" are excluded and that misconduct means the fabrication, falsification and plagiarism of data.

The final rule retains the draft proposal that an institution must be able to inquire "immediately" into an "allegation or other evidence of possible misconduct" and complete its inquiry within 60 days. If a full investigation is found necessary, it must begin within 30 days and be completed within a further 120 days.

Extra guidance is given on the way an investigation is to be performed. The final rule requires "examination of all documentation, including . . . research data and proposals, publications, correspondence and memoranda of telephone calls". All involved are to be interviewed and summaries of what they said given to them for "comment or revision". And the rule helps whistleblowers by adding a requirement for "diligent efforts" to be made "to protect the positions and reputations of those persons who, in good faith, make allegations". The draft version provided only for the reputations of those "alleged to have engaged in misconduct when allegations are not confirmed".

The final rule deliberately does not deal with measures "to foster scientific integrity" and lists, as issues that remain to be addressed, the retention of laboratory data, authorship practices ['ghost' and multiple authorship], and the performance of audits or studies to prevent scientific misconduct.

Alun Anderson

Fretting about the future

Munich

RECEIVING an offer to become director of a Max Planck Institute is still the dream of many researchers in and out of West Germany, and no wonder: no teaching responsibilities, a lifetime position and the opportunity to do research without having to apply for grants are among the privileges a director enjoys. But members of the Max Planck Society (MPS) have become concerned that the high scientific standards of the illustrious society might start to slip.

The MPS scientific council sounded an alarm earlier this summer with a two-day symposium on "Generational dynamics and innovation in basic science". The symposium did more to identify disturbing questions concerning the future of MPS than to answer them.

On the one hand, the lower ranks of MPS are filling up with people who have lifetime tenure. But in the next few years, MPS will have to appoint dozens of new directors to replace those who will be retiring. MPS is now asking itself if its current structure is worth maintaining.

MPS has achieved its status in part because of its unusual structure. Because a director with a lifetime appointment is not required to publish, he or she can choose a project that takes many years or that requires special training. That allows for the type of project that led to the 1988 Nobel prize in medicine or physiology (awarded to Hartmut Michel, Robert Huber and Johann Deisenhofer who did the work at the MPS Institute for Biochemistry) — an interdisciplinary project that takes several years and calls for researchers with highly specialized capabilities.

As an indicator of its success, MPS has lured numerous German researchers back home from good overseas posts; Stanford University and the biotechnology company Genentech have recently lost senior people to MPS.

The principle upon which the predecessor of MPS, the Kaiser Wilhelm Society (KWS), was founded in 1911 still affects life in MPS today. The 'Harnack principle', named after Adolf von Harnack, a theologian and a founder of KWS, called for a scientific institute to be built around a leading personality in science, where the person could be surrounded by subordinates of his or her choosing.

The principle has admittedly been tempered in MPS (which was set up in 1948) by the practice of having several directors in a single institute. In many cases, an institute has department heads

who form an executive board that makes decisions on a majority basis. But the biochemistry institute in Martinsried is more typical. There, each of the 11 directors has his own budget, with autonomy over decisions on personnel and laboratory space. The director is thus an absolute monarch within his own department.

It is this freedom that makes an MPS directorship such a dream — but von Harnack certainly never reckoned with the impact of West German labour law on scientific flexibility. When a director retires from a Max Planck Institute, it can in theory be shut down and its resources given to a new director in a different field. In practice, however, each director leaves large groups of employees behind.

These people, as qualified as they might be, can be a millstone around the neck of the director's successor, if his or her research takes a new tack. Under West German law, these employees may not be made redundant.

The next director is required to find work for them until they retire, which can often take ten to twenty years. When the present MPS president Heinz Staab took office in 1984, roughly 75 per cent of MPS scientific personnel had lifetime positions. Staab considered this a handicap for MPS in its search for the best new directors and he urged that more people



Staab of MPS: Sees little prospect of reducing the number of lifetime tenure posts.

be hired on short-term contracts. But in five years, the number of staff with lifetime positions has fallen little and Staab says he does not see how it could be reduced much more in the foreseeable future.

Many of the recent problems in MPS can be traced to the sclerosis that has gripped West German universities in the 1980s. Formerly, even subordinate researchers with lifetime positions could be counted on to leave MPS for a university job when the right offer came along. Now, there is a jam at the top of the steep pyramid that is a West German university, so these offers come rarely. By contrast, a

US university faculty is structured "more like an inverted pyramid, with most positions at the highest level", said Harvard sociologist Aage Sorensen at the symposium.

Sociologist Hans Joas of the University of Erlangen pointed out that West German universities need a more complete career ladder, complete with middle rungs. Otherwise, the boom-or-bust cycle for young researchers that began in the generous university expansion of the 1960s will continue. Research administrators all over West Germany are asking themselves how to break this cycle. For MPS, some think the large turnover at the top will be good — it "will only increase the scientific standard", says Ken Holmes, an MPS director in Heidelberg. But efforts to bring in and hold onto the best people will have to be redoubled when the logjam at universities breaks.

Otherwise, says another director, these same people may jump at university jobs, leaving behind the 'dead wood'. In the opinion of Jeff Schatz, a biologist at the University of Basel who has served on two MPS advisory boards, MPS has made one of its best contributions to West German science by establishing small institutes such as the Friedrich Miescher Laboratory in Tübingen.

The great advantage of the Miescher laboratory is its policy of "five years and out", which Staab says is enforced flexibly but firmly to keep up the flow of new people. In the coming years, said Staab, new laboratories will be installed exclusively for junior researchers in biology-related institutes in Cologne, Berlin, Freiburg and Martinsried. But the number of young people thus employed will be only a tiny fraction of the overall MPS payroll of 2,400 scientific positions.

Despite the symposium, radical change within MPS seems unlikely. Once a director is appointed, he or she usually does not push very hard to change the system that is supporting him so generously. One person who did try to change things, Nobel laureate Georges Köhler of Freiburg, learned quickly that the MPS administration has a strong interest in defending the *status quo*. Köhler, who has been at MPS for five years, suggested that middle-level positions be created and that young researchers be offered the opportunity to stay at MPS. His suggestion fell on deaf ears. "It is my impression that the administration wanted to maintain the current system because it is easier to administer", he says.

But Staab is stepping down next year, and the selection of a new president may bring about a reassessment. Trying to shake a society burdened by its own custom and by external conditions will not be easy; but the next president, who will be in office until 1996, may by then have no choice.

Steven Dickman

Understanding of science

SIR—Durant, Evans and Thomas (*Nature* **340**, 11–14; 1989) rightly point out that public understanding of science is essential in a highly technological democracy. But they do not address the question of whether our representatives understand science any better than the general public. When will they apply their test to Members of Parliament (MPs)? If we can be guided by the experience of the United States, where a test a few years ago showed that 60 per cent of US senators could not name five African countries (including such obscure ones as South Africa), the results may be rather depressing: what hope for sensible legislation on CFC emissions or spare-part surgery if nearly 200 MPs believe the Sun goes round the Earth?

WILLIAM BAINS

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SIR—At first sight, it was surprising and depressing to read that 30 per cent of 2,000 members of the British public questioned thought that the Sun went round the Earth. But then I remembered the words of Dr Thomas Arnold, who was headmaster of Rugby School in the 1830s and who is often described as the founder of the modern British public school. He feared that physical science was such a large subject that it would dominate the school curriculum, to the exclusion of the inculcation of Christian, moral and political philosophy. He knew where his priorities lay. In a letter to a friend, he said: "Rather than have physical science the principal thing in my son's mind [his son was Matthew Arnold, later to be an inspector of schools as well as a poet], I would gladly have him think that the sun went round the earth and the stars were so many spangles set in the bright blue firmament".

DAVID DAVIES

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SIR—Here are two very recent quotations on a similar theme: (1) "Nuclear power stations are a cause of acid rain", source—nearly half of Britons surveyed by Durant *et al.*, and (2) "Nuclear power is irrelevant to the prevention of global warming", source—100 of the country's leading scientists doctors and engineers (Greenpeace declaration, most daily papers, 5 July 1989).

These two quotations provide a neat complementarity of misinformation;

about SO₂ and NO_x emissions by the first group and CO₂ emissions by the second group. This is dispiriting to scientists involved with investigating causes and consequences of acid rain and the greenhouse effect. Let us hope that as the science proceeds to dispel some of the scientific uncertainties, the standard of debate will also take an upturn.

MAX BERAN

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Events in China

SIR—Like many others, I have been saddened and appalled by recent events in China and have wondered what can be done to register a meaningful protest. I think there is one way that scientists can have an impact. Many organizations have planned meetings and conventions in China that would bring significant amounts of hard currency into the country. These meetings should be cancelled and re-scheduled elsewhere. How can we attend meetings in a country where our colleagues are under arrest, where too much fraternizing with foreigners can compromise them, perhaps leading to punishment of them or their families after the meeting is over?

Only by widespread, concerted action will such a protest have an effect; I urge all who are planning to hold or attend meetings in China in the near future to consider their colleagues—some now dead or under arrest—and to decide whether this is an appropriate time to be carrying on business as usual.

RAYMOND S. BRADLEY

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Fraud in science

SIR—I agree with Efraim Racker (*Nature* **339**, 91; 1989) that fraud in science is ugly, but there are always three parties involved: the victim (the scientific community), the offender and his boss. We all know what may happen. The "unbalanced mind" working at the "forefront" is told by his supervisor that he has an idea which, if proved, will be a "breakthrough". What that will mean is clear. This is the beginning. No bad guy, no good guy. Just someone who hears what he wants to hear and someone who supplies. The pressure may come from within but this does not exonerate the supervisor from responsibility.

Fraud in science is not a political but a moral question. But it is first a moral

question for the supervisor. Co-workers working for their doctorate are very dependent. So fraud in science is also the deception of beginners by established scientists.

THOMAS FRIEDRICH

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Science and slides

SIR—Scientists use slides as an important way to exchange information at scientific meetings. Modern computerized techniques in general lead to better slides. Nevertheless, on almost every occasion we experience the frustrations of failing slide projection. One common problem is the mismatch between the thickness of the slides and the corresponding part of the slide projector. I advise US colleagues not to bring slides that are too thin when they come to Europe. Conversely, the thinner slides work better for Europeans when we go to the United States. And, of course, we should all leave paper-framed slides at home.

BEN DE KRUIJFF

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Dispute resolved

SIR—This letter is intended to put an end to a dispute concerning speculative models for the possible role of reverse transcription in:

- (1) the production of antigen-directed mutations in the antibody genes of antigen-stimulated B lymphocytes (Steele, E.J. & Pollard, J.W. *Molec. Immun.* **24**, 667–673; 1987);
- (2) the production of substrate-directed mutations in certain bacterial genes (Cairns, J., Overbaugh, J. & Miller, S. *Nature* **335**, 142–145; 1988).

These speculations had their origin in Temin's ideas about the possible role of retroviruses and reverse transcription in the genesis of biological variation (Temin, H.M. *Perspect. Biol. Med.* **14**, 11–26; 1970 and *J. Natn. Cancer Inst.* **46**, iii–vii; 1971).

One of us (E.J.S.), in a letter he distributed to many people, has claimed that the other (J.C.) was guilty of plagiarism. He now acknowledges that the allegation was unfounded and he withdraws it.

E. J. STEELE

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J. CAIRNS

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Too few foreign scientists in Japan

Shahid S. Siddiqui

International exchange between Japanese and foreign researchers would benefit both groups. Why are foreign scientists still so reluctant to work in Japan? And what awaits them there?

THERE has never been a time like this for foreign researchers to come to Japan. Hundreds of postdoctoral fellowships have recently become available at Japanese universities and research laboratories. In 1982, a law was passed to allow foreign nationals to assume full-time faculty positions at the national and public universities. Yet foreign researchers from the United States and other Western countries are not coming to Japan in the numbers expected.

Japan's Science and Technology Agency (STA), for example, has advertised a new scheme for 100 postdoctoral fellowships per year; half reserved for the US researchers and the rest divided among ten countries and the EEC. The conditions of these fellowships are very generous: they range from 6 months to 2 years and provide a tax-free monthly stipend of ¥270,000 (\$2,000) plus allowances. Another 100 similar postdoctoral fellowships are available in universities, financed by the Ministry of Education, Science and Culture (MESC), and administered by the Japanese Society for Promotion of Science (JSPS). As a result of Western pressure, particularly from the United States, together with Japan's desire to improve its scientific image, the government has greatly increased outlays for basic research and international exchange. Under these attractive conditions, what prevents young US and other European researchers from coming to Japan?

There are several immediately obvious answers, including cultural differences, lack of job security, isolation from the West, the rigid hierarchical system of Japanese institutions, poor research funding, and a general apprehension among foreign researchers that they have little to gain in Japan due to the type of research conducted there. Some of these notions are based on realities, others derived from misconceptions and for historical reasons.

Traditionally, Japan has been an importer of science and technology from the West. The fast rise in the standard of living since the ruins of the Second World War was achieved by sending Japanese people to the United States and elsewhere to acquire new skills in science and technology and to learn large-scale production processes. But there was little interest in basic research in Japan at that time. Over the years, the exchange of scientists between Japan and the Western countries has been a one-way street. In 1983, for

example, 24,000 researchers left Japan to work in technologically advanced nations whereas only 5,000 went in the opposite direction. By contrast, about 5,000 Japanese researchers went to work in technologically less-developed nations, from which 24,000 researchers arrived in Japan.

Nevertheless, Japan has undergone an unprecedented growth in industry and technology since the Second World War. It has earned worldwide respect and envy, and is headed for a leading role in the world economy. This prosperity has led to the notion that Japan is all out to export consumer goods, and Japanese science is perceived to be entirely devoted to the task of 'picking winners' in science and technology.

This is true to a large degree, but is not the entire picture. A recent survey¹ shows that, during 1973–82, Japan increased its proportion of scientific publications by 4.9% per year, far exceeding the relative figures for the United States (0.5%), the United Kingdom (–0.8%), West

Germany (0.8%) and France (–0.3%). A similar conclusion is reached by Garfield², who analysed national publication productivity for the years 1973–82 and concluded that Japanese scientists increased their output by about 38%. Although it is difficult to assess the standard of Japanese science according to the numbers of publications, it is evident that there is growing interest in basic research.

Recruitment

Part of the problem in the recruitment of foreign scientists may lie in poor publicity. The JSPS-sponsored fellowships for UK scientists were undersubscribed for years until publicity from the Royal Society led to the number of applicants rising until the scheme is now oversubscribed by more than two to one.

In principle, faculty positions are advertised through the job circular (*Ippan Kobo*) displayed on the notice boards in national and public universities and other institutes, and potential candidates can

TABLE 1 Numbers of foreign faculty members at national universities and institutions in Japan

University/Institute	Professor	Associate professor	Lecturer	Total
Hokkaido Univ.	1			1
Hokkaido Kyoiku Univ.		1		1
Hiromae Univ.		1		1
Iwate Univ.			1	1
Tohoku Univ.			3	3
Ibaraki Univ.	2	1	4	7
Tsukuba Univ.		4	2	6
Chiba Univ.		1		1
Tokyo Univ.	2	3	1	6
Tokyo Gaigo Univ.		2		2
Tokyo Kogyo Univ.		1	1	2
Yokohama Kokuritsu Univ.		1		1
Shinshu Univ.			1	1
Nagoya Univ.		1		1
Toyohashi Tech. Univ.	1	3		4
Shiga Ika Univ.			1	1
Kyoto Univ.	1		1	2
Kyoto Kogyo Univ.	1	1		2
Osaka Univ.		3	2	5
Kobe Univ.		1		1
Okayama Univ.			1	1
Hiroshima Univ.	1	3	3	7
Yamaguchi Univ.		1	1	2
Kagawa Univ.		1		1
Ehime Univ.	1			1
Kochi Ika Univ.			1	1
Kyushu Univ.	1	3	1	5
Kyushu Geikou Univ.	1			1
Saga Univ.	2	1	2	5
Oita Univ.		1		1
Oita Ika Univ.			1	1
Kagoshima Univ.		1		1
High Energy Research Institute		1		1
Museum of Ethnology		2		2
International Centre for Japanese Culture	1			1
Total numbers	15	38	27	80

Data provided by MESC (1 July 1988).

apply from anywhere. But in reality, the common practice is appointment by nomination (*Enko*). In Japan, it is very important for a job applicant to be properly 'introduced', or recommended, for a position. This does not mean that those hired are incompetent, but nevertheless, the right connections are critical when the competition for jobs is severe.

The competition for academic positions stems from a chronic lack of new positions in the universities. During the 1960s there was a sudden expansion of the universities, resulting in many PhD graduates but no jobs for them to fill, mainly because there are not postdoctoral positions and

number of foreign faculty members is 80, compared with 34,938 Japanese faculty members. Obviously these low numbers will not induce other foreign researchers to join faculty positions at the Japanese universities. Another problem is that many foreign faculty members do not remain in their posts for long, as shown in Table 2.

There is a growing dissatisfaction with the *status quo*, and there are signs that the rigid structure of the Japanese universities may change. MESC is supporting new universities that are innovative and free from convention. In 1976, for example, two new universities were established to

societies. Peer review, therefore, is restricted to a handful of scientists, who are burdened with hundreds of applications.

One of the sections in the grant application form deals with the relevance of the proposal in relation to similar studies being conducted abroad. This may be a recipe for inadvertently copying Western science, discouraging original ideas and suppressing creativity in research. Foreign scientists are also expected to write grant applications in Japanese, and in the absence of money to hire secretaries they rely on help from their co-workers to translate for them.

It may be wise for MESC to provide 'special funds' to foreign researchers to establish their research laboratory to attract good scientists. When I joined the faculty of the Toyohashi University of Technology in 1986 my task was to create a laboratory of molecular biology with start-up money of ¥4 million (\$30,000). The budget crisis was acute. The first relief came in the form of a competitive grant from MESC of about ¥7.5 million (\$56,250) for 3 years. Investment in the universities is gradually improving, as MESC is encouraging universities to receive funds from industry and donations. For example, our laboratory has received research funds from the NEC Corporation and Kowa Pharmaceutical Company. The procedure for accepting donations is, however, very bureaucratic.

Future prospects

Despite many social and cultural differences, most foreigners become accustomed to life in Japan, and even enjoy it. But if Japan is seriously trying to recruit more foreign researchers, it will have to deal with the problems they face, including housing, language, schools, cost of living and travel allowances, otherwise most of the goodwill generated by a warm welcome to Japan evaporates. This having been said, the Japanese try very hard to make the life of foreigners comfortable, and the rest is up to the visitor.

Science has no international boundaries — scientists from different nations should share experiences, and compare approaches and techniques. There is no doubt that an increase in international exchange between Japanese and foreign scientists would benefit not only the individual scientists but also the countries involved. □

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1. Smith, D.C. *et al.* *Nature* **323**, 681–684 (1986).
2. Garfield, E. *Curr. Cont.* **9**, 3–9 (1987).
3. Yamamoto, A. *Nature* **339**, 575–576 (1989).

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TABLE 2 Pattern of employment of foreign faculty members at the national universities and institutes in Japan

Year	Professor			Associate professor			Lecturer			Total		
	Hired	Left	Total	Hired	Left	Total	Hired	Left	Total	Hired	Left	Total
1982												
1983	2		2	6		6	6	1	5	14	1	13
1984	1		1	8	1	7	1	1	0	10	2	8
1985	4		4	6	2	4	4	2	2	14	4	10
1986	2	2	0	5	3	2	7	2	5	14	7	7
1987	12	4	8	11	6	5	13	5	8	36	15	21
1988	1	1	0	14		14	8	1	7	23	2	21
	22	7	15	50	12	38	39	12	27	111	31	80

Data provided by MESC (1 July 1988).

because the structure of the faculty is very rigid (see ref. 3). Research assistants have to wait years to be promoted: at present there are more than 17,000 assistants working in universities, many of them in their 40s.

Before 1982, no foreign national was allowed to assume a faculty position in the national and public universities in Japan. This changed in 1982, when a law was passed permitting foreigners to be appointed at these universities: "any foreign faculty member, shall not be prevented from being a member of a faculty meeting, or any other consulting bodies organized for the operation of the university for the sole reason that he or she is a foreign national". As for the term of appointment: "the term of the service of the foreign faculty member shall be decided by the governing authority of the particular university".

The term of appointment for foreigners, in general, varies between 1 and 5 years, and can be renewed. Foreign scientists who come to Japan and wish to stay for 1 to 2 years would need job security. In practice, however, it is very difficult if not outright impossible for a foreign national to get tenure similar to a Japanese colleague. The tenure of a Japanese scientist is not affected by his work, and his salary is commensurate with his seniority. This apparent discrimination may inhibit many foreign researchers.

Table 1 shows the number of foreign faculty members at the national and public universities since 1982. It is remarkable that only 32 of the 95 national universities have any foreign faculty members. Furthermore, as of July 1988, the total

introduce more flexibility in the existing university structure: Toyohashi University of Technology and the Technological University of Nagaoka. Students come from the colleges of technology (*Kosen*), and are admitted to the third year of the university. The universities offer undergraduate and postgraduate courses that are consistent with their education and training. At the Toyohashi University of Technology, some of the lectures are given in English, a unique event for Japanese universities.

Research funds

It may seem ironic that in Japan, which ranks next to the United States in funding research, the universities are so poorly funded as the bulk of the research money is in the industrial sector¹. In fact, the universities are worse off now than 20 years ago, as funding has not kept pace with inflation². Even when MESC does provide a large sum of money to a university or a research institute, the money is provided only for the construction of the facility, or buying equipment, and none is provided to create positions.

The nice thing about competitive grants in Japan is that the application forms are only five or six pages long, unlike the United States where such applications are usually very long. But in Japan no critique of the grant proposal is provided to the applicant: either it is approved, or he receives a note of regret, without information about the strengths and shortcomings of his proposal (there are very few women scientists in Japan). Typically, a review committee consists of three members, who are nominated by scientific

Basic research comes alive in Japan

The richest country in the world seems to have acquired a broadened basis for basic research that promises both serious competition with the West and a distinctive style.

Tokyo

NOBODY seems to have a clear view of the bearing of last week's dramatic upheaval in the politics of Japan on the future of basic science, even if the continued prosperity of Japanese technology seems assured. Part of the uncertainty arises because the opposition parties that secured two-thirds of the vacant seats in the Upper House of the Japanese parliament differ among themselves on questions such as the future role of nuclear power or on the protection of the environment, and in any case have said very little about the function of basic science in the Japanese scheme of things.

If there is a common theme in what the opposition parties say other than their view that corrupt governments are undesirable, it is that they abhor the new 3 per cent consumption tax. But the chances are that the ruling Liberal Democratic Party, frightened by the election result, will move quickly to modify the tax, making it less unpalatable. In principle, that could affect the government's willingness to spend public funds on the improvement of research support, especially in the universities. But the general opinion is that the bureaucrats who run the ministries have acquired such a taste for improvement, and are in any case so powerful, that the cheerful tendencies of the past few years can only continue.

For decades, it has been a glaring paradox that Japan, while heading for unparalleled and inimitable prosperity, should have so neglected its universities and their capacity for research. Now that Japan is literally the richest country (with a gross domestic product of \$23,000 per head of population in 1988), the signs of neglect are still apparent in the older buildings. But the idea that things are on the mend is almost palpable.

All this is most obvious in people's demeanour. One noticeable change is that the once-familiar excess of politeness has melted away, to be replaced by a more general courtesy. Even the practice of thrusting a visiting-card (called a name-card) at every visitor is no longer universal (and some apologize for not having one). The result is that it is easier to hold straightforward conversations even about questions which are largely Japanese (such as the future of the government). To say that people are more confident of themselves in relation to their competitors in the West would be demeaning, and probably incor-

rect as well. Rather it is that there appears to be many more active people with a detailed knowledge of and curiosity about what similar groups elsewhere are doing.

There have also been institutional changes. In some fields, high-energy physics, for example, the recent successes of national research programmes seem to have had an almost tangible effect on people's morale. The electron accelerator at the Tskuba laboratory called KEK is a proof not merely that Japanese can build big machines, which was hardly ever in doubt, but that they can use them for sound physics as well. In the same way, people are delighted that the Japanese neutrino detector seems to have functioned as well as that of the predominantly Irvine-Brookhaven collaboration in detecting a bunch of neutrinos from the supernova 1987A.

That, no doubt, is why some at least of the physics community are resentful of the rumours widely circulating that, as part of the negotiations between the United States and Japan on their disputed trading relationship, Japan is being asked both to contribute to the cost of the Superconducting Super-Collider to be built in Texas and to commit the Japanese community to detectors for that machine. At least some people say they would prefer Japan to invest in the European machine (LEP) at Geneva.

US negotiators may not appreciate how much resentment they have engendered over issues such as these; to point out that the Government of Japan spends a smaller proportion of its income on basic research than does the government of the United States may be correct, but to imply that natural justice requires more spending in Japan, and to go on to specify how a substantial number of Japanese should occupy themselves, seems tactless, to say the least.

Other institutional changes could have an even more profound influence on the future. Among other things, a group of national laboratories led by the three national research institutes at Okazaki have successfully created their National Graduate University — a degree-giving institution now boasting of nearly 50 PhD students. In the years ahead, there is bound to be intensifying rivalry between this institution without a central campus, dependent as it is on recruitment of students from the 96 national universities and the national universities themselves.

Among academics proper, there is much unfamiliar talk of mobility and diversity. At least at the universities of Tokyo and Kyoto, it seems to be agreed that it will be neither feasible nor desirable simultaneously to improve the status of research at each of the national universities. That is why people at these institutions are full of praise for schemes being devised by bureaucrats at the education ministry (almost affectionately known as Mombishu) for imparting a sense of competition to the annual pursuit of financial support.

It is true that one such device, Mombishu's scheme for awarding large research grants worth up to the equivalent of \$1 million a year too often go to predictable safe hands and, sometimes, are spent on over-ambitious projects. (But Japan would not be what it is without a measure of calculated recklessness.) Similarly, the national graduate university is welcomed (but not at Tokyo, which has ambitions to assume the role itself) as a means by which able young people from all parts of the national university system will be put into general academic circulation.

Meanwhile, some universities have found ways round the straitjacket of uniformity Mombishu traditionally imposes on itself. Kyoto has, for example, used private funds to create two new institutes on its campus — one to span the interface between computer science and psychology — and, with Osaka, is well launched on a scheme to found an Institute of Advanced Study 500 kilometres away from Tokyo.

However the funds arise, there seems no doubt that the pattern of academic research in Japan will be distinctive. On successive mornings last week, I visited the Seiko company's development laboratory, complete with its automatic watch-production line being used to train the managers of a new plant in Singapore, and the national laboratory called RIKEN, founded in 1917 as a means of improving Japanese science and technology. The styles are virtually identical. Young men (as yet, there are few women) are in charge of their own projects and proudly explain them, however halting their English. The two laboratories seem identical in their readiness to tackle difficult tasks. But it is no longer just a matter of making machines function: people seem to know clearly what they want them to accomplish.

John Maddox

Genetic linkage revisited

William F. Byerley

MUCH enthusiasm in the field of schizophrenia research was engendered when Bassett *et al.*¹ reported that partial trisomy of a region of chromosome 5 (20–30 centimorgans at 5q11.2–13.3) cosegregated with schizophrenia in two members of a family of Asian descent. Chromosomal aberrations, although not responsible for the genetic transmission of diseases in general, can provide clues to where diseased genes lie and in the event that the location of such a gene is established, these accidents of nature can facilitate cloning of mutant alleles². With the lead provided by Bassett *et al.*, Sherrington and associates³ found preliminary evidence of linkage between 5q11–13 and schizophrenia in seven families. At the same time, Kennedy *et al.*⁴ published data suggesting that this genomic region was not linked to schizophrenia in a large geographically isolated kindred. Now at least two other research groups^{5,6}, one on page 391 of this issue, report their failure to find linkage between 5q11–13 and schizophrenia, casting some doubt on the finding of Sherrington *et al.*. Where does this leave the case of linkage of schizophrenia to 5q11–13?

Penetrance values

Sherrington *et al.* identified five Icelandic and two English families with multiple cases of schizophrenia in two or more generations and found a segregation pattern of schizophrenia in the families that appeared to be compatible with autosomal dominant transmission with reduced penetrance. They excluded for study any families with bipolar disorder (manic-depression), a prudent decision because family, twin and adoption studies indicate, in general, that schizophrenia and bipolar disorder are genetically unrelated. The families include 39 cases of schizophrenia, 5 cases of schizophrenia spectrum disorders (illnesses that may represent attenuated forms of schizophrenia) and 10 cases of fringe phenotypes such as alcoholism, phobic disorder, minor depressive disorder and major depressive disorder (illnesses that are usually caused by other factors and that are unlikely to be alternative manifestations of a schizophrenic gene).

After genotyping the families with two polymorphic DNA markers localized to 5q11–13, Sherrington *et al.* carried out multiple analyses in which phenotypic status and disease penetrance were varied. Somewhat surprisingly, they found evidence of linkage across all analyses. Using relatively high penetrance values (73–86 per cent), Sherrington *et al.* derived maximum lod scores of 3.22 (for

individuals with schizophrenia), 4.33 (for those with schizophrenia and schizophrenia spectrum disorders) and 6.49 (for schizophrenia, schizophrenia spectrum disorders and fringe cases).

It is not surprising that evidence of linkage increased when the phenotype definition was broadened to include cases of schizophrenia spectrum disorders, because family, twin and adoption studies suggest that these disorders are alternative expressions of the schizophrenia gene, but it is hard to reconcile the observation that the lod score increased when the fringe phenotypes were included as affected cases. In general, evidence of linkage should decrease when the phenotypic definition of the illness is enlarged to include less certain cases, as the addition of just a few false-positive cases (dependent somewhat on the position of these cases in the pedigree) in a linkage analysis can significantly lower lod scores.

One might, at least in hindsight, question the use of relatively high penetrance values for a psychiatric disorder. This may have given undue weight to the information provided by unaffected individuals, falsely elevating lod scores. When studying a disease with variable penetrance and expressivity, it may be best to use a very conservative analytical scheme in which only strictly defined cases are considered affected. Sherrington *et al.* undertook such an analysis and derived a maximum lod score of 2.45. Given that there was an *a priori* hypothesis of where the gene is located, this lod score may be statistically significant, even though a score of 3 or more is the conventional criterion for linkage. Although it is often assumed that a lod score of 3 yields a 1,000:1 odds in favour of linkage, in actuality the probability is closer to 20:1 because there is a one in fifty chance that any two randomly chosen genetic loci are localized to the same chromosomal region.

In summary, the study of Sherrington *et al.* still seems promising but additional work is needed before firm conclusions can be made. Assuming for the moment that true linkage has been found, it is likely that the dominant allele on chromosome 5 is responsible for only a subset of genetically-based schizophrenia and as many as 50–200 additional pedigrees may need to be studied before linkage to 5q11–13 is found in other families.

In contrast to Sherrington *et al.*, Kennedy and associates did not find evidence of linkage between chromosome 5q11–13 and schizophrenia in a Swedish pedigree. Of all the known schizophrenic pedigrees being studied, this is one of the most optimal for linkage analysis because the

family has many individuals with schizophrenia as defined by strict diagnostic criteria, as well as low levels of drug and alcohol abuse and no apparent cases of bipolar disorder. As the family is geographically isolated, and as the pedigree seems to be segregating mental retardation in addition to schizophrenia, this family may be afflicted with a relatively unusual genetic form of schizophrenia.

Transmission

Although Kennedy *et al.* presented evidence against linkage of schizophrenia to chromosome 5 markers, the significant negative data were based on analyses assuming autosomal dominant transmission and a relatively high penetrance (72 per cent). Transmission of schizophrenia in the kindred, however, may be more compatible with autosomal recessive inheritance or autosomal dominant transmission with low penetrance. If autosomal recessive inheritance is operating in the family, then lack of linkage to the putative dominant allele on chromosome 5 is to be expected. If autosomal dominant inheritance with low penetrance is responsible for transmission of disease in the family, then the preferred analysis may be of affected cases only, because a penetrance of 72 per cent may have incorrectly assigned a number of unaffected individuals as cases. It is true, as the authors point out, that no evidence of linkage was found even when lower penetrance values were assigned, but use of penetrance as a parameter and the risk of even small errors in phenotyping make it easier to reject than detect linkage.

Two other groups have recently also reported exclusion of schizophrenia to 5q11–13, but 8 of the 15 families studied by St. Clair *et al.*⁵ and 2 of the 5 pedigrees investigated by Detera-Wadleigh and associates in this issue⁶ had cases of bipolar disorder. It is conceivable that these families are segregating two genes — one for bipolar disorder and one for schizophrenia — a circumstance that would complicate phenotypic determination as well as linkage analysis. As a result, the negative linkage data reported by these two groups must be viewed with caution. St. Clair *et al.* did report the results of a linkage analysis between one chromosome 5q marker and schizophrenia in the six families without cases of bipolar disorder, but these data (a lod score of –2 or more at 10 per cent recombination) do not sufficiently exclude a genomic region that spans 20–30 centimorgans.

Although these conflicting reports may have caused some disillusionment in the field, there is room for optimism. The use of computer programs⁷ that can analyse the inheritance of a number of loci simultaneously and the availability of a high-resolution restriction fragment length polymorphisms linkage map should help

identify genetic loci causing schizophrenia in pedigrees not linked to 5q11–13. (To search systematically for a mutant allele with the linkage map, one may need to analyze the inheritance of 200–400 markers; because multiple analyses will increase the probability of false findings, it may be wise to increase the lod score taken to indicate linkage.) The resolution of the map linkage continues to improve and may now be of the order of five centimorgans between markers, although considerable gaps exist in some genomic areas. More high-quality DNA markers, most notably variable number of tandem repeat markers, are being identified and used to construct linkage maps⁸. Such markers will render more families informative for linkage analysis and increase

the likelihood of detecting diseased genes. Within the next few years, the goal is to construct a one-centimorgan linkage map, which will greatly facilitate gene identification and cloning. Thus, the tools that may help elucidate the genetics and neurobiology of schizophrenia are at hand. □

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1. Bassett, A.S., McGillivray, B.C., Jones, B.D. & Pantzar J.T. *Lancet* **1**, 799–801 (1988).
2. Orkin, S.H. *Cell* **47**, 845–850 (1986).
3. Sherrington, R. et al. *Nature* **336**, 164–167 (1988).
4. Kennedy, J.L. et al. *Nature* **336**, 167–170 (1988).
5. St. Clair, D. et al. *Nature* **339**, 305–309 (1989).
6. Detera-Wadleigh, S.D. et al. *Nature* **340**, 391–393 (1989).
7. Lathrop, G.M. et al. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3443–3446 (1984).
8. Nakamura, Y. et al. *Science* **235**, 1616–1622 (1987).

EARTH SCIENCES

Hot plumes from the mantle

K.G. Cox

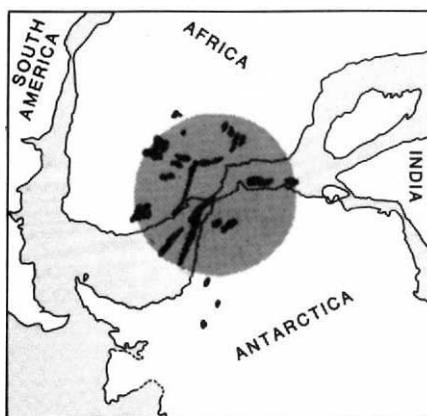
Two new articles^{1,2} by R.S. White and D.P. Mackenzie of Cambridge University under the modest titles of 'Magmatism at Rift Zones' and 'Volcanism at Rifts' present one of the most satisfying and unifying geological syntheses to have appeared in recent years.

Every 30 million years or so for at least the past 250 million years, continental areas have been subjected to enormous volcanic episodes, generating so-called continental flood basalt provinces, including those of the North Atlantic, Ethiopia, Deccan, Karoo and Paraná (the last exemplified by the spectacular falls on the Rio Iguazu depicted on the cover of this issue). The authors develop a model explaining such provinces in terms of abnormally hot convective jets (plumes) rising from the Earth's mantle, and undergoing decompressive melting as the crust and lithosphere, the outermost rigid shell of the Earth, is stretched. Continental rifting, continental break-up, the formation of new oceans and the uplift of continental land-masses outside mountain belts, are all phenomena that are readily explained in terms of the model. The model also has profound implications for the growth of continental crust through geological time.

The point about this particular model is that although the jets rising from the depths are narrow (about 100 km across), they develop a wide mushroom top in which mantle rocks 100–200 °C above normal mantle temperatures may spread out to underlie an area perhaps 2,000 km in diameter (see figure). The dynamic effect of the plume is calculated as producing uplift of the surface of up to 2 km in the centre of a broad dome. Gravitational forces tend to produce rifting of the dome, and if plate tectonic forces are favourable (that is, the continent is already under

extensional stress) the arrival of such a plume from below will have dramatic consequences.

A new ocean will be formed as the continent breaks, and huge amounts of basaltic magma will be produced by melting owing to the coupling of the excess mantle temperature with decompression resulting from the mantle rising to fill the void. Some of the magma produced is erupted at the surface to form continental flood basalts and thick sequences of lavas along the margins of any new ocean that is



The extent of Late Jurassic basaltic volcanism in southern Africa and the Dronning Maud Land area of Antarctica (black ornament) with the continents in their pre-drift positions. The shaded circle shows the postulated extent of the top of the Karoo plume (redrawn from ref. 2).

formed. A significant fraction, however, is trapped below the continental crust (the phenomenon known as underplating) which increases crustal thickness and in the long term may be a significant component of crustal growth. Isostatic adjustment causes the surface of the continent to rise so that the initial topographic dome is

maintained even long after the plume has expired or the continental masses concerned have drifted far away from the plume.

Clearly the model has significant implications for our understanding not only of rifting and magmatism but also for many features of the geology of within-plate regions, especially the uplift of continental areas outside mountain ranges, which does not find any simple explanation in terms of plate tectonics.

One is forced, however, to ask first, what is new, and second, is it just another idea that owes more to imagination than science, for example like some recent proposals that continental flood basalts were generated by meteorite impacts? With regard to novelty, it is true that most of the ideas incorporated, for example the plume concept and the association of plumes with volcanism, rifting and uplift, have been familiar for quite a long time. The work does indeed have many of the elements of a review, but it is one in which the whole is greater than the sum of the parts. It is the proposed extent of the plume tops and their high temperatures that have enabled the authors to integrate such a wide range of large-scale geological phenomena into a single simple structure.

The model represents the fusion of a number of quite different strands of research, some of which have been pursued patiently, but not particularly dramatically, for decades. The geological, geochemical and geochronological description of continental flood basalt provinces has occupied many research workers for a long time, which is hardly surprising considering their sheer size. A second ingredient has been the long-term study of the structure and stratigraphy of continental margins and the geomorphology of continental areas, with implications for uplift and subsidence. The synthesis of experimental data on the phase relations of basaltic rocks by McKenzie & Bickle³ marked an essential step in the argument, by making possible the incorporation of melt volumes and compositions into geophysical models of mantle convection and thermal structure. An important final ingredient was White's earlier seismic work in marine geophysics⁴ from which the realization came that seaward-dipping reflectors off continental margins probably represent huge extensions of the adjacent continental basaltic provinces, thus contributing to the idea that if plumes were involved they had to be a good deal bigger than most people had previously thought.

The authors' thorough assessment of a very large amount of geological literature contributes to my feeling of confidence in their conclusions, though in detail there is some tidying-up to do. I think the Karoo plume probably has to be bigger than

presently envisaged. I am worried that the plume that generated the Walvis Ridge in the South Atlantic does not appear to have been anywhere near the centre of what I take to be the topographic uplift in the adjacent continents. The Transantarctic Mountains have so far not been included in the model at all, and their exclusion from the Karoo plume on the grounds of geochemical difference is hardly fair, because many of the geochemical characters of continental flood basalts are not related to the plume itself but are generated by interaction with lithospheric

rocks. These are not important criticisms, merely indications of some of the large number of exciting problems that can now be attacked within the unifying framework which has been presented. □

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1. White, R.S. & McKenzie, D.P. *J. geophys. Res.* **94**, 7685–7730 (1989).
2. White, R.S. & McKenzie, D.P. *Scient. American* **261**, 62–67 (1989).
3. McKenzie, D.P. & Bickle, M.J. *J. Petrol.* **29**, 625–679 (1988).
4. White, R.S. *Nature* **327**, 191 (1987).

TRANSPORT PROTEINS

Export-import family expands

Chris Higgins

WHAT is the connection between such topical subjects as yeast mating type, resistance of human tumours to chemotherapy, protein secretion, bacterial transport and the failure of chloroquine as an antimalarial drug? Whereas this question sounds obscure enough to be borrowed from *Round Britain Quiz*, the answer is readily apparent from two new papers^{1,2} including that by McGrath and Varshavsky on page 400 of this issue². Each process involves a member of a family of closely related proteins, all of which are believed to be associated with membranes and to bind ATP. These proteins have now been identified in bacteria, yeasts, protozoans, insects and mammals and their similarities imply that each functions by a rather similar mechanism.

Perhaps the most familiar member of this family is the P-glycoprotein or MDR (multidrug resistance) protein. Overexpression of MDR protein in mammalian tumours is responsible for the simultaneous development of resistance to a variety of apparently unrelated chemotherapeutic drugs. The MDR protein consists of two hydrophobic domains, each of which includes about six potential membrane-spanning helices, and two hydrophilic ATP-binding domains^{3–5}. All available evidence is consistent with the view that MDR protein pumps drugs out of cells, reducing their intracellular concentration and, hence, their toxicity. The intriguing similarity in sequence and domain organization between MDR protein and a group of well-characterized, ATP-dependent proteins that are known to be involved in the transport of small molecules across bacterial cell membranes implies that MDR functions by a similar mechanism^{6–8}.

Three differences do, however, exist: the domains of the bacterial transport systems are present as separate polypeptides rather than a single multifunctional polypeptide like MDR protein; the bacterial systems transport substrate into the cell while MDR protein pumps substrate out-

wards; and the bacterial systems require an additional protein (the periplasmic binding protein) to deliver substrates to the membrane-associated proteins. These differences probably reflect diversification of a single common transport mechanism.

Just as tumour chemotherapy is hindered by multidrug resistance, so the control of malaria has been impeded by the development of chloroquine resistance (CQR) in *Plasmodium falciparum*. CQR shares many of the characteristics of MDR found in multidrug resistance in tumours and Foote *et al.*¹ have recently isolated a gene from *P. falciparum* that encodes a homologue of the MDR protein. Not only does the protein encoded by the *P. falciparum* gene have a very similar sequence and domain structure to MDR protein but its gene is amplified in several chloroquine-resistant cell lines, just as *mdr* is amplified in multidrug-resistant tumour cell lines. It seems reasonable to assume that the CQR protein pumps chloroquine out of parasite cells much as MDR protein pumps drugs out of tumour cells. Although, of course, there are many transport systems in both eukaryotes and prokaryotes that rely on quite different proteins, it seems that the MDR and CQR proteins and a group of bacterial transporters comprise a single family of transport proteins. Other members of this family may include the *white* and *brown* loci of *Drosophila*, which are required for the laying down of eye pigments and are related by sequence to *mdr*.

Many questions remain unanswered. Most importantly, it is entirely unclear how the MDR protein exhibits specificity for such a diverse range of substrates when, in bacteria, highly specific transport systems exist for each individual substrate to be transported. Related to this is the question of the identity of the physiological substrates and normal cellular roles of the MDR and CQR proteins. Drugs such as Adriamycin or chloroquine

are clearly not normally encountered by the cells and for MDR, it is clear that simple amplification of the normal protein is sufficient to confer resistance⁹. For CQR, it remains to be established whether simple amplification is responsible for resistance or whether the protein must be modified by mutation to enable it to handle chloroquine. The fact that CQR is stably inherited suggests that more than simple amplification may be involved.

In the elegant and intriguing paper in this issue², McGrath and Varshavsky for the first time establish the normal cellular function of a eukaryotic member of the MDR protein family. However, rather than ending speculation, their unexpected findings serve to emphasize how little we actually know. They demonstrate that the *STE6* gene of yeast, which is essential for the export of a-factor mating pheromone from the cell, encodes an MDR homologue. Although it remains a formal possibility that the inhibition of a-factor export is an indirect effect of *STE6* mutations, the authors suggest that *STE6* protein does, itself, mediate a-factor export by a process that differs from that used to secrete most yeast proteins. This raises many questions. Why should a-factor require a different export mechanism? Is *STE6* used to export other proteins? And is *STE6* the only protein required for the process to operate? Intriguingly, the HlyB protein of *Escherichia coli* required for haemolysin secretion shares considerable sequence similarity with both *STE6* and the MDR protein, particularly in the ATP-binding domains, although the hydrophobic domains do differ somewhat, and, unlike the mammalian proteins, HlyB has just a single hydrophobic domain and a single ATP-binding domain^{5,6}.

So, we have a rather unexpected paradox. MDR and many of its homologues in eukaryotes and prokaryotes appear to mediate the transport of small molecules across the membrane whereas other members of the family appear to mediate translocation of specific proteins. These two processes, while superficially the same, have generally been considered to involve entirely distinct mechanisms. This assumption may now need re-examination. Many surprises are likely to be in store. □

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1. Foote, S.J., Thompson, J.K., Cowman, A.F. & Kemp, D.J. *Cell* **57**, 921–930 (1989).
2. McGrath, J.P. & Varshavsky, A. *Nature* **340**, 400–404 (1989).
3. Gros, P., Croop, J.M. & Houseman, D. *Cell* **47**, 371–380 (1986).
4. Chen, C. *et al.* *Cell* **47**, 231–239 (1986).
5. Gerlach, J.H. *et al.* *Nature* **324**, 485–489 (1986).
6. Higgins, C.F. *et al.* *Nature* **323**, 448–451 (1986).
7. Higgins, C.F. *et al.* *BioEssays* **8**, 111–116 (1988).
8. Ares, G. *et al.* *Cell* **47**, 323–324 (1986).
9. Gros, P., Neriah, Y.B., Croop, J.M. & Houseman, D.E. *Nature* **323**, 728–731 (1986).

Electrically heated asteroids

William B. McKinnon

THAT the asteroid belt is broadly arranged by spectral type with distance from the Sun has been known for over a decade. Now new observations and improved compositional interpretations of these spectral types have better characterized the belt as a function of both heliocentric distance, as shown in the figure, and asteroid size. These in turn suggest to Herbert, in a recent paper¹, that the primordial thermal evolution of the asteroids was driven by electromagnetic induction in a 'T Tauri' type wind erupting from the young Sun.

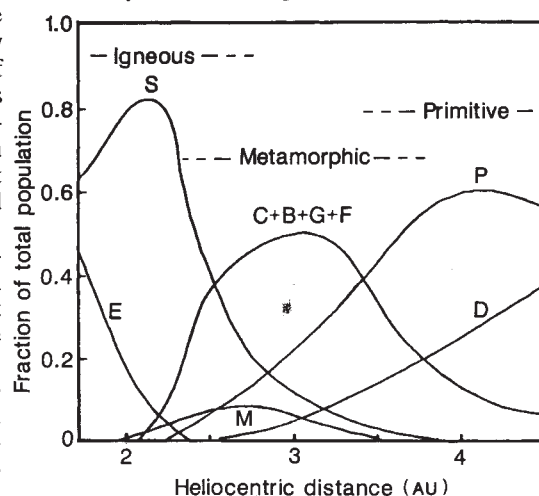
The evolutionary history of the asteroids — how they formed and how they were subsequently altered mineralogically — is an important part of the history of the whole Solar System, and contains vital clues to the processes that operated. The notion that asteroid alteration resulted from induction heating is not new, but it seems in rather good accord with the emerging picture of belt history.

The figure shows the bias-corrected distribution of the principal asteroid types and their present compositional interpretations. The S, M and E spectral (taxonomic) types all show spectral or other evidence for igneous minerals and in some cases nickel-iron. The abundant S types, in particular, are inferred to be the partially exposed metallic cores of differentiated asteroids². The M types are inferred to be more completely exposed cores or core fragments. These asteroid classes are common in the inner-to-mid asteroid belt, but virtually absent in the outer belt.

Type C and similar types are grouped together and believed to be related to carbonaceous chondrite meteorites, generally considered 'primitive'. These asteroids occupy the middle belt and are generally characterized by very low albedos, spectral neutrality and an absence of absorption features. The P and D types are also of very low albedo, but have generally red slopes in the visible and near-infrared. They are characteristic of the outer belt, with the redder D-types predominating in the Trojan clouds, off the figure at 5.2 astronomical units. No meteorite is thought representative of the P or D asteroids, and P- and D-asteroid materials have occasionally been dubbed ultracarbonaceous.

The concept of primitiveness has now been revised. The most volatile-rich carbonaceous chondrites are now recognized as being thoroughly (if not completely) altered by low-temperature aqueous solutions (below 25–150 °C). Hence they are not primitive, but metamorphosed. Fur-

thermore, spectrophotometric observations of the dark asteroids show evidence for water of hydration on many of the C asteroids (about two-thirds of those studied), but not on the distant P and D asteroids³. A plausible explanation is that during the condensation and accretion of the solar nebula in the asteroid region, the kinetics of gas-solid reactions were too slow for hydrated silicates and other hydrous minerals to form⁴. Asteroids then accreted from a mixture of ice, organic and anhydrous silicate grains, a mixture not unlike that thought to compose comet Halley and to be represented in part by



Distribution of asteroid compositional types versus heliocentric distance (adapted from ref. 1 and based on unpublished estimates of C. R. Chapman).

interplanetary dust particles. Moderate heating released water to hydrate the silicates, form other low-temperature phases such as carbonates and sulphates, and promote complex organic reactions — the combination resulting in C-type asteroids. Asteroids in the outer belt remained unheated for reasons suggested below; so, provided any surface ice was lost to sublimation (or impact processing) over the age of the Solar System⁵, their surface minerals could be anhydrous.

The asteroid belt can apparently be divided into three broad zones (see figure). Moving inward, asteroids pass from being truly primitive, to moderately metamorphosed, to igneously differentiated. Catastrophic disruption events have also contributed to the mix of types by exposing different levels within individual parent bodies, and gravitational scattering has broadened the zones occupied by each asteroid type. A natural inference to draw is that a heat source highly dependent on heliocentric distance operated in the asteroid belt, provided that the present distribution of asteroid types was created roughly in place. Herbert¹ points out that

when the bias-corrected asteroid types are sorted by size, the igneous classes seem to cluster at intermediate sizes (less than 50 km diameter). He interprets this to mean that the primordial heat source was also a function of asteroid size, an intermediate size being favoured.

A dense, powerful wind is thought to have been generated by the forming Sun, as happens in young variable 'T Tauri' stars. Electrical induction driven by this wind would give a heat source with the required characteristics for the asteroid classes. Analogous to the induced currents driven through Io's ionosphere by Jupiter's corotating magnetospheric plasma, this phenomenon could have efficiently coupled the kinetic energy flux of the primordial solar wind to the deep interiors of proto-asteroids. The induced fields are strongest close to the Sun, of course, and their effect is reinforced by a dependence of electrical conductivity on temperature such that warmer asteroids (larger ones and those closer to the Sun) are heated more efficiently. Moreover, magnetic deflection of induced currents reduces heating in the largest bodies, so there is a preferred body size for maximum temperature rise.

The induction-heating model has a number of free parameters — the duration and intensity of the dense solar wind and asteroid electrical conductivity — so in principle one can arrive at any answer one wishes. Nevertheless, Herbert shows that a plausible T Tauri wind lasting about 10^5 years, and a temperature-dependent electrical conductivity similar to that of the CM carbonaceous chondrite Murchison, can reproduce both the spatial and size distribution of heating inferred from asteroid compositional types. In general, measurements of the high-temperature electrical conductivity of carbonaceous meteorites show both high values and a strong temperature dependence⁶, possibly related to organic phases⁷, making the electrical-induction hypothesis all the more plausible.

Herbert further notes that neither the size- nor the distance-dependent variations in asteroid heating are obviously explained by the oft-invoked primordial heat source, radioactive ^{26}Al . The initial abundance of ^{26}Al in asteroids is unclear. Excess radiogenic ^{26}Mg (derived from the decay of ^{26}Al) was found previously only in refractory inclusions; but recently, when an excess was reported in a chondrule in an ordinary chondrite⁸. Herbert also argues that there is no evidence connecting ^{26}Al with the thermal metamorphism of any known meteorite and, by implication, with any parent asteroid. Nevertheless, the ^{26}Al issue is not settled, and thermal evolution models based on this heat source are being pursued⁹.

What the recent work on asteroid

taxonomy, compositions, evolution and induction heating shows that a plausible and interesting framework exists to explain the diverse observations. Further work is obviously necessary. For example, can an asteroid actually be heated in the manner proposed? As practical experience with microwave ovens suggests, heating may take place in preferential regions, may run away owing to temperature-dependent conductivity effects, or may even short through. Furthermore, contrary to the dominant idea at present, the current distribution of asteroid types may not reflect the original distribution. The S and M types may have been injected from the region of the inner planets and the D asteroids may be outer-Solar-System planetesimals (or even comets) that have migrated inward. The possibility of cometary-type orbits evolving to those resonant with Jupiter has been demonstrated theoretically¹⁰. A fundamental issue to resolve is whether the asteroid belt was a dumping ground for diverse

early Solar System bodies, or whether it marks the limit of the Sun's major control over protoplanet composition and thermal evolution. □

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1. Herbert, F. *Icarus* **78**, 402–410 (1989).
2. Gaffey, M.J. *Icarus* **60**, 83–114 (1984).
3. Lebofsky, L.A. & Jones, T.D. *Lunar planet. Sci.* **XX**, 562–563 (1989).
4. Prinn, R.G. & Fegley, B. in *Origin and Evolution of Planet and Satellite Atmospheres* (eds Atreya, S.K., Pollack, J.B. & Matthews, M.S.) (University of Arizona Press, Tucson, in the press).
5. Lebofsky, L.A., Jones, T.D. & Herbert, F. in *Origin and Evolution of Planet and Satellite Atmospheres* (eds Atreya, S.K., Pollack, J.B. & Matthews, M.S.) (University of Arizona Press, Tucson, in the press).
6. Duba, A.G. & Boland, J.N. *Lunar planet. Sci.* **XV**, 232–233 (1984).
7. Duba, A.G. & Shankland, T.J. *Geophys. Res. Lett.* **9**, 1271–1274 (1982).
8. Hutcheon, I.D., Hutchison, R. & Wasserburg, G.J. *Lunar planet. Sci.* **XX**, 523–524 (1988).
9. Grimm, R.E. & McSween, H.Y. *Icarus* (in the press).
10. Milani, A., Carpino, M., Hahn, G. & Nobili, A.M. *Icarus* **78**, 212–269 (1989).

ANTHROPOLOGY

Were Neanderthals the first humans to bury their dead?

Jared M. Diamond

THERE have been many reports that Neanderthals ritually buried their dead. Particularly striking are claims of skeletons associated with apparent remains of features such as flower offerings, burial pits, circles of goat horns and evidence of bear cults. If correctly interpreted, these graves would constitute the first evidence in human evolution not only for burial of the dead, but also for spiritual beliefs. Robert Gargett, however, concludes in a recent report that these observations do not prove the point — but there are several rebuttals accompanying his paper (*Current Anthropology* **30**, 157–190; 1989). The debate, far from being of purely romantic interest, is central to understanding the origins of 'humanity'.

Gargett's objections to the evidence are that features attributed to intentional burial may be natural, that evidence for association of supposed grave offerings with skeletons is weak or lacking, and that interpretations of rituals go beyond the available facts. As he remarks, for example, "holes in the ground are not necessarily the result of human digging"; they can arise in many natural ways. Horizontal and vertical stones near a bear skeleton at Regourdou cave were originally interpreted as remains of a carefully constructed coffer with pavement and wells, but stones falling naturally from a cave's roof could have come to rest at these angles. Skeletons flexed as if in sleep have been taken to indicate deliberate

burial, but are consistent with natural death during sleep, as is surely true of recently discovered flexed mummies of two Eskimos who had been crushed by ice falling on their house. The flowers whose pollens and anthers at Shanidar Cave have been interpreted as remains of flower offerings may have been blown into the cave's large mouth, been brought by nesting rodents, or have simply grown there. At Teshik-Tash, where a Neanderthal skeleton was described as lying within a circle of goat horns placed point down, there are actually six nearby horns of which the positions of only two suggested point-down placement. Most bones in the cave were of goats (brought by carnivores?), so that chance could explain six of the durable horns being preserved near the human skeleton.

The published responses to Gargett's article by 11 groups of authors cover the whole spectrum, from qualified agreement ("a very well founded and sound reinterpretation"; A. Weber) to scathing disapproval ("We have difficulty finding any scientific merit in this paper"; D. Frayer and A. Montet-White). Some responses concede that the older excavations discussed in detail by Gargett are inadequate, but maintain that more recent excavations are more convincing. Frayer and Montet-White, Gamble and Trinkaus identify a key problem: why are human skeletons in Europe before the last interglacial so fragmentary, and why do

nearly complete skeletons not appear until the time of the Neanderthals in the last glacial?

If burial did not occur, why are some Neanderthal skeletons found with fragile elements intact, with bone frequencies as in modern cemeteries, and with anatomically natural positions, particularly as animal skeletons at similar sites of that period are not preserved in this way? Gargett responds to their question by suggesting there was better preservation of the more recent human skeletons because of the gradual natural disruption of older skeletons, and that Neanderthals, unlike animals, lived and died in caves that are favourable sites for preservation.

The contrasting views of Gargett and his critics address a wider issue. Neanderthal sites have yielded no convincing evidence of paintings, sculpture, engravings, jewellery, long-distance trade, formal bone tools, or marked geographical or temporal variation in stone tools. Nor do sites of the earliest anatomically modern *Homo sapiens* from southern Africa more than 100,000 years ago. All these features, as well as unquestioned burials and grave goods, appear with or soon after the arrival of anatomically modern *H. sapiens* in Europe around 35,000 years ago. Thus, anatomy that was virtually modern — at least insofar as it can be judged from skeletons — led to modern behaviour only after a long period of further development. If skeletal anatomy was not the explanation, what else caused this great leap forward in behaviour, and where and how did it suddenly arise?

Here the argument about burials becomes crucial. If Neanderthals did ritually bury their dead, they had at least one type of spiritually motivated behaviour that allies them with modern humans rather than with animals. If they did not, then perhaps they should be regarded as little more than technically skilled chimpanzees with control of fire and monotonously unvarying stone tools. In the latter case, it would be easier to understand why there is no convincing evidence for hybridization between Neanderthals and anatomically modern *H. sapiens*.

The debate between Gargett and his critics shows that these issues now merit re-investigation. Potential natural explanations for Neanderthal 'burials' should be scrutinized; animal and human skeletons likely to have become buried naturally need to be compared with intentionally buried human skeletons to provide better criteria for identifying intentional burials; and detailed accounts of St Césaire and other recent Neanderthal excavations need to be published. Until then, the question of whether Neanderthals buried their dead remains contentious. □

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Metallic hydrogen and beyond

N.W. Ashcroft

Has hydrogen finally been converted by pressure into a metal, on a laboratory bench? Perhaps, is the cautious but potentially vivifying experimental answer given recently by Mao and Hemley in *Science*¹. And will it be, after all, a high-temperature superconductor? Very much so, is the confident answer given by Barbee, Garcia and Cohen in a theoretical paper on page 369 of this issue².

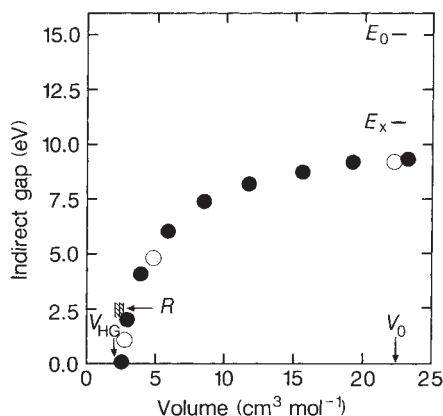
More than 50 years ago it was first predicted by Wigner and Huntington³ that at sufficiently high densities and correspondingly high pressures, the molecular bonds in dense hydrogen⁴ would be ruptured, the bonding electrons liberated, and the entire assembly transformed into a monatomic metal, much like an alkali metal. But according to their estimates, to achieve such a state would require static compression by almost a factor of 10 in density, corresponding to pressures in excess of 2 million atmospheres. The problem of 'metallizing' hydrogen has remained an experimental challenge of the first rank. Remarkably that challenge has gradually been faced and met over the years, principally through the invention and systematic development of the diamond anvil cell⁵.

Although diamond itself will ultimately share the same fate as hydrogen, in the pressure range typical of a million atmospheres it remains transparent and provides a window, literally, through which optical probes for the highly compressed hydrogen can pass. Mao and Hemley exploit this window in two different ways using optics with especially high spatial resolution. First, they monitor the optical behaviour of hydrogen as a function of pressure, also obtaining estimates of its reflectivity. This information is linked in a reasonably direct way to the behaviour of hydrogen's electronic properties within an independent-electron description.

Second, Mao and Hemley conduct measurements where small shifts in the wavelength of the probe light are observed. These manifestly inelastic processes (the Raman effect) give information on the dynamics of the protons, principally the relative motion of protons within molecular pairs (the intramolecular vibron). Because the coupling of light to these degrees of freedom is mainly indirect, actually proceeding via the electrons, this kind of measurement may also give corroborative information on the way the electronic structure is changing with pressure.

The Raman effect is also capable of revealing structural changes, where the shifts in wavelength are found to change discontinuously with pressure. In an earlier paper⁶, Hemley and Mao observed

just such a shift occurring at a pressure estimated to be 1.5 million atmospheres. (H. E. Lorenzano *et al.* have recently confirmed this; personal communication.) And in just the past few months (personal communication) Mao and Hemley have seen a corresponding shift at approximately 2.0 million atmospheres in deuterium, the mass-2 isotope of hydrogen. Though some differences arising from the *ortho-para* (nuclear symmetry) nature of



Predicted indirect gaps in hydrogen (in the $Pa3$ or $\alpha-N_2$ structure) as a function of molar volume. Points from Friedli and Ashcroft⁸ (○) and Min *et al.*¹⁵ (●). The volumes V_0 and V_{HG} are respectively the molar volume of solid hydrogen at one-atmosphere, and the volume at which the Hertzfeld–Goldhammer criterion predicts the onset of the metallic state. The indirect gap is different from the direct optical gap, E_0 , for volume V_0 ; it also differs from the measured exciton energy E_x . Both E_0 and E_x are expected to fall with increasing pressure. The area indicated by R locates an approximate gap inferred by Mao and Hemley from trends in the intensity of the Raman spectrum. As can be seen it appears to be in line with the progression of the indirect gap which in turn suggests that primary optical activity either involve phonon assistance (in which case the absorption should be temperature dependent) or must involve the free charge in a band overlap state.

the states are to be expected, these results indicate strong quantum or zero-point effects from proton or deuteron motion as well as the appearance of new structures, so far unknown, at very high pressure.

In their *Science* article¹, it is the pressure region above this structural transition which is of special interest; in particular Mao and Hemley observe that above 2 million atmospheres, hydrogen dramatically but continuously changes from a clear solid to one acquiring a quite dark appearance. Correspondingly, there is a noticeable change in the intensity of the Raman scattering. The inference they make is appealing; interband transitions between electronic bands are occurring in the visible part of the spectrum (1.7–3.0 electron

volts). It would follow that the large band-gap characteristic of the insulating solid phase at one atmosphere has been drastically lessened by application of pressure. Indeed, if it were to close entirely, then in principle a semi-metallic state could be achieved. Within an assumption that diatomic order is preserved, which from the very existence of an observed vibron is reasonable, this 'band-overlap' mechanism to the formation of a semi-metallic state is an alternative route to metallization, not foreseen at the time of Wigner and Huntington's paper.

So the crucial issues centre on whether at these enormous pressures the band gap has completely closed and whether ground-state free charge exists. Put in another way, is hydrogen in this pressure range behaving like a narrow-gap semiconductor (like silicon, perhaps) or like a semi-metal (say arsenic)? And is it possible to distinguish visually between these alternatives in samples only a micrometre or so thick? (Even these two examples do not entirely exhaust the range of possibilities: it is known on general grounds that a band gap cannot be closed continuously. Going beyond the independent-electron view, the condensation of an 'excitonic' fluid is another candidate.) With respect to the existence of mobile charge, the most desirable tests, namely the direct measurement of conductivity or, less directly, using far infrared reflectivity, are both difficult to carry out with dense hydrogen in a diamond cell. It is also prudent to keep in mind that because of large stress gradients, the hydrogen samples are really quite non-uniform and some aspects of heterogeneous or composite behaviour in these cells must therefore be inevitable.

On the positive side, however, the projected vanishing of the gap at about the measured pressures is generally supported not only by band-structure calculations^{7,8} but also by the partially empirical Hertzfeld–Goldhammer criterion (based on divergence of molar refractivity) which is fairly successful in predicting metallization in other systems. Though band gaps are not usually well given by local density approximations, the trend according to band theory is shown in the figure, with the α -form of solid nitrogen as the chosen structure. It is important to note that what is plotted is an indirect gap (for most reasonable structures, direct gaps would be much larger). Because the absorption of light across such a gap can proceed only with the aid of phonons (by definition), the darkening observed by Mao and Hemley should be temperature dependent.

Failing this, optical activity related to free charge becomes a more promising possibility. However, much of this is conditional on knowledge of the structure: as noted, this is not known. Accordingly, Mao and Hemley's identification of the

onset of a metallic state has to remain tentative. Nevertheless the issue might soon be resolved because in a tantalizing penultimate comment, Mao and Hemley report that their apparatus is capable of reaching pressures approaching 3 million atmospheres. This is squarely in the range of current estimates of the pressure required to achieve the dissociated (Wigner–Huntington) phase. In a related and certainly most eye-catching statement, they remark that even in the present series of experiments there seems to be, in the very darkest hydrogen regions, a notable absence of the key signature of the molecule, namely the vibron.

It is this fully dissociated state that Barbee *et al.* treat in their discussion, in this issue², of high-temperature superconductivity, a possibility first ventured more than 20 years ago. (Even earlier, some general remarks had been made by Abrikosov⁹ on the likelihood of superconductivity in highly compressed matter.) Although the concept also extends to the metallic but paired form of metallic hydrogen¹⁰, most theoretical treatments of this problem have concentrated on Wigner and Huntington's view of the metallic state as a monatomic phase. Several calculations exist in the literature of the superconducting transition temperature and this is often placed in the range of 100–200 K, impressively high even compared with the newer oxide superconductors.

One of the most detailed treatments to date is the essentially first-principles calculation by Whitmore *et al.*¹¹, who showed that very high superconducting transition temperatures are possible in hydrogen but who also warned that the results are very structure dependent. This seems to be borne out by the paper of Barbee *et al.* who examine an unusual structure, a distorted hexagonal lattice originally proposed by Brovman *et al.*¹². (Hexagonal structures appear, from past work¹³ to be relatively favourable from the standpoint of high-temperature superconductivity, possibly because they give rise to some low-lying phonon modes.)

In their calculation, Barbee *et al.* use more recent band-structure methods to establish the electronic structure of hydrogen in this phase. By imposing small structural distortions on the system (so-called frozen phonons) they can even estimate, from the consequent changes in energy, the lattice vibrational energies at a few chosen wavevectors. Though this is a far from complete phonon spectrum, sufficient dynamical information is available for them to obtain a plausible estimate of the average electron–phonon–electron coupling required in the expression for the transition temperature (either the McMillan form, or by direct solution of the Eliashberg equations for strong-coupling systems).

It has to be recognized that, over the years, the predictive ability of superconductivity theory has had a chequered record. Barbee *et al.* come up with an estimate for the transition temperature which is in the neighbourhood of 200 K, and in support of this calculation they cite a previous success with the group-IV element, Si, which also becomes a metal at higher densities, although at much more moderate pressures. It is surely gratifying that silicon does become a superconductor at high pressure; nevertheless, in view of hydrogen's position in the periodic table, perhaps a better comparison might be with the group-I elements, the alkali series. These are already metals at 1 atmosphere, and among the series lithium, with its very small core, is probably the closest analogue to metallic hydrogen. To date, lithium, in its bulk form, has not been found to be superconducting. This is very much at odds with theory which, with techniques very similar to those used by Barbee *et al.*, predicts that it should be (and with reasonable transition temperatures).

This discrepancy is disturbing. Perhaps the resolution lies in the warning about structure given by Whitmore *et al.*¹¹, for it is now known that the light alkali metals have low-temperature structures that are extremely different from their familiar high-temperature cubic forms. These are rhombohedral, but interestingly enough they can be viewed as a stacking sequence of hexagonal layers with a ninefold repeat. It is possible that metallic hydrogen will take up such a structure; if so, it begins to look not terribly different from the distorted hexagonal form that Barbee *et al.* prefer. Continuing in this vein, if metallic hydrogen adopts such a phase (and Barbee *et al.* say that 4 million atmospheres are needed to produce it) but is found not to be superconducting, then given the simplicity of this system and the fact that at a very basic level the interactions are known exactly, the question may then begin to shift somewhat to the

theory of superconductivity itself. The main issue will be the presumed ubiquity of the electron–phonon–electron pairing mechanism which has prevailed since the earliest prediction for metallic hydrogen, as it has for most systems until the recent discovery of the oxide superconductors.

There have been many claims in the past of the successful metallization of hydrogen, but none so far passing the absolute requirement of verifiable reproducibility. The problem remains an important one because of the fundamental significance of hydrogen and because as a metal it is exceptional in possessing no bound or localized electrons. In fact, the problem of producing metallic hydrogen is cited by Ginzburg¹⁴ as one of the key problems in physics and astrophysics. Also on his list is high-temperature superconductivity, so this single system presents both in propitious combination. At the very least, the recent experimental advances of Mao and Hemley and other groups also working on dense hydrogen indicate that this long-sought state cannot be far off. □

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1. Mao, H.K. & Hemley, R.J. *Science* **244**, 1462–1465 (1989).
2. Barbee, T.W., Garcia, A. & Cohen, M.L. *Nature* **340**, 369–371 (1989).
3. Wigner, E. & Huntington, J. *J. chem. Phys.* **3**, 764–770 (1935).
4. Silvera, I. *Rev. mod. Phys.* **52**, 393–452 (1980).
5. Jayaraman, A.J. *Scient. Am.* **250**, 54–62 (1984).
6. Hemley, R.J. & Mao, H.K. *Phys. Rev. Lett.* **61**, 857–860 (1988).
7. Ramakr, D.E., Kumar, L. & Harris, F.E. *Phys. Rev. Lett.* **34**, 812–814 (1975) C.
8. Friedli, C. & Ashcroft, N.W. *Phys. Rev.* **B16**, 662–672 (1977).
9. Abrikosov, A.A. *JETP* **14**, 408–416 (1962).
10. Ashcroft, N.W. *J. phys. Chem.* **156**, 41–51 (1988).
11. Whitmore, M.D., Carbotte, J.P. & Shukla, R.C. *Can. J. Phys.* **57**, 1185–1195 (1979).
12. Brovman, E.G., Kagan, Yu. & Kholas, A. *JETP* **34**, 1300–1315 (1972); **35**, 783–787 (1972).
13. Schneider, T. & Stoll, E. *Physica* **55**, 702–710 (1971).
14. Ginzburg, V.L. *Waynflete Lectures on Physics* (Pergamon, Oxford, 1983).
15. Min, B.I., Jansen, H.J.F. & Freeman, A.J. *Phys. Rev.* **B33**, 6383–6390 (1986).

PLANT EVOLUTION

A high mutation rate in a long lived perennial plant

Deborah Charlesworth

DISADVANTAGEOUS mutations are suspected to be very important in evolution, for example in the maintenance of outbreeding sexual reproduction and genetic recombination¹, in the evolution of aging² and in the genetic inactivation of Y-sex chromosomes^{3,4}. Mutation can cause genetic variation in fitness in populations⁵, and much of the lowering of fitness on inbreeding is probably caused by partially recessive mutations with minor effects⁶.

These theories have not yet been empirically tested to any great extent. On page 389 of this issue Klekowski and Godfrey demonstrate that data on mutation rates can be obtained from natural populations.

We have little knowledge of mutation rates in natural populations, and even less on the magnitude of mutational effects on fitness. Rates obtained from domesticated species may be higher than for natural

populations because of artificial selection. Maize, for example, has been selected for kernel phenotypes caused by mobilization of transposable elements. Mutation rates from laboratory organisms such as mice are usually for single loci. These are biased towards loci with high rates, which not only require the smallest number of individuals to be studied, but are also disproportionately represented among known loci for higher organisms, because loci mostly became known only when a phenotypic difference caused by a mutation is detected.

Single-locus mutation rates are also of limited interest because many of the evolutionary models in which mutation is important are framed in terms of deleterious mutation rates for the whole genome¹. Estimates from natural populations, using unbiased sets of loci, together with estimates of the distribution of effects of mutations on fitness in both the homozygous and heterozygous states, are needed. These are unattainable at present, but some useful information can be obtained in certain favourable cases, such as the work of Mukai on viability in *Drosophila melanogaster*^{6,8} and in the paper by Klekowski and Godfrey⁷ on a mangrove species *Rhizophora mangle*.

The method used by Klekowski and Godfrey for mangroves is often used in human genetics, and is known as the indirect method of estimating mutation rates, because new mutations are not detected directly. Rather the mutation rate is estimated indirectly from genotype frequencies in a population assumed to be at equilibrium, with the number of new deleterious mutations arising each generation at a locus exactly balanced by the loss of mutant alleles due to their effects on fitness⁹. Klekowski and Godfrey studied mutations in chlorophyll-deficient (white or yellow) seedlings. Mangroves have the convenient property that seedlings are borne on maternal plants for long periods of time and can be scored at this stage. Mother-offspring sets are thus obtainable, unlike the situation in most organisms living in the wild.

R. mangle seems to be highly self fertilizing: in most cases, individuals bore either a high proportion of chlorophyll-deficient seedlings — close to a quarter — or none at all, as would be expected on selfing a heterozygote if the phenotype were due to recessive alleles. The family sizes were big enough that there should be little ascertainment bias owing to the fact that ratios could be recorded only for maternal plants bearing at least one abnormal seedling. Klekowski and Godfrey found various different phenotypes, showing that the estimates of mutation rates they obtained are not for just a single locus but for a sample of loci in *R. mangle* that can mutate to give chlorophyll deficiency.

Klekowski and Godfrey generalize the

indirect method for estimating rates of mutation to recessive lethal alleles to the case of complete selfing. Populations with partial selfing could also be dealt with, using the formulae of Ohta and Cockerham¹⁰. From the frequencies of heterozygous maternal plants, Klekowski and Godfrey estimate mutation rates for two mangrove populations to be 7.4×10^{-3} and 5.9×10^{-3} , very similar values. The fact that different populations yield similar estimates of the mutation rate lessens the possibility that the rates were wrongly estimated due to the heterozygote frequencies being far from the equilibrium value.

A problem could arise, however, from the assumption that heterozygotes for the mutant alleles have the same fitness as wild-type homozygotes⁸. If they had slightly lowered fitness, as the data for recessive lethals from *Drosophila*⁶ and maize¹¹ indicate, the true mutation rate would have to be higher than estimated to produce the same heterozygote frequency, whereas if they had higher fitness than wild-type homozygotes, there would be an overestimate. It seems unlikely that recessive lethal alleles could be maintained by heterozygote advantage in populations of such a highly inbreeding species as *R. mangle*. There have, however, been reports of heterozygote advantage for chlorophyll-deficiency alleles in some species¹².

Although the mutation rates for *R. mangle* are very high, they are probably underestimates rather than overestimates, because heterozygotes are not detected unless a homozygous mutant progeny is produced. This problem is not too serious

for a highly self-fertilizing species if large numbers of progeny are scored, but could be serious if a partially selfing plant were studied. Klekowski and Godfrey conclude that the mutation rate in *R. mangle* is higher than for plants with shorter life-spans, although they do not mention the very high value (1.3×10^{-4}) directly estimated for *Mimulus guttatus*, an annual or short-lived perennial¹². The idea that long-lived plant species have high mutation rates because of the greater number of cell divisions before gamete formation is consistent with the evidence that mutation rates and rates of molecular evolution are higher in male mammals than in females, where there are more cell divisions in sperm formation than in female gamete production^{13,14}. □

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1. Kondrashov, A. *Nature* **336**, 435–440 (1988).
2. Medawar, P.B. *Modern Quarterly* **1**, 30–56 (1946).
3. Charlesworth, B. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5618–5622 (1978).
4. Rice, W.R. *Genetics* **116**, 161–167 (1987).
5. Charlesworth, B. in *Sexual Selection: Testing the Alternatives* (eds Bradbury, J.W. & Andersson, M.) 1–40 (Springer, Berlin, 1987).
6. Simmons, M.J. & Crow, J.F. *A. Rev. Genet.* **11**, 49–78 (1977).
7. Klekowski, E.J. & Godfrey, P.J. *Nature* **340**, 389–391 (1989).
8. Mukai, T. *Genetics* **61**, 749–761 (1969).
9. Haldane, J.B.S. *Int. Congr. Genetics, Stockholm. Hereditas suppl.* 267–273 (1949).
10. Ohta, T. & Cockerham, C.C. *Genet. Res.* **23**, 191–200 (1974).
11. Crumpacker, D.W. *Evol. Biol.* **1**, 306–423 (1967).
12. Kiang, Y.T. & Libby, W.J. *Am. Nat.* **106**, 351–367 (1972).
13. Miyata, T., Hayashida, H., Kuma, K., Mitsuyasu, K. & Yasunaga, T. *Cold Spring Harbor Symp. quant. Biol.* **52**, 863–867 (1987).
14. Dryja, T.P. *et al. Nature* **339**, 556–558 (1989).

MINERALOGY

Carbon dioxide in the deep crust

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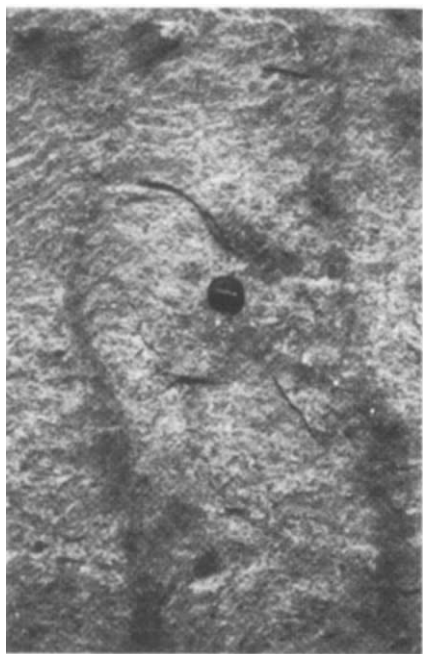
It is not only in the atmosphere that the effects of carbon dioxide are a cause for both debate and concern. It has been known for some time that the mineral assemblages we see in metamorphic rocks often have stabilized when in equilibrium with an unseen fluid phase, but it has been much harder to demonstrate the source or estimate the flux rates of crustal fluids. Opinions vary between those who favour a fluid-absent lower crust and those who prefer a lower crust infiltrated by substantial volumes of mantle-derived CO₂ (ref. 1). On page 378 of this issue², Peterson and Newton present new experimental evidence that suggests that CO₂ will reduce the temperature at which common crustal rocks will begin to melt. One important implication is that if CO₂ has degassed from the upper mantle into the lower crust it may have allowed partial melting of the deep crust, thereby affecting both the evolution of crustal melts and

the chemistry of the lower crust.

When crustal rocks are sufficiently hot, common crustal minerals — such as micas and amphiboles — dehydrate; the resulting anhydrous assemblages are known as granulites. Because in general the deeper rocks are in the crust the hotter they become, granulites characterize much of the deep crust. Granulites, however, are not formed only at the deepest levels of the crust, because mineral assemblages can dehydrate at moderate temperatures and pressures in the presence of a water-poor fluid phase, such as a CO₂-rich fluid.

Metamorphic petrologists working on granulites have searched for evidence of a CO₂-rich fluid in their formation for a decade or more. Fluid inclusions found in many granulites are known to be both abundant and rich in CO₂ (ref. 3), but fluid inclusions can be entrapped late in the rock's history and therefore be unrelated to granulite formation⁴. Strong evidence

of an active role for CO₂-rich fluids in granulite formation comes from south India where the irregular relationship between hydrous and anhydrous assemblages is clearly fluid controlled⁵ and often the fluids can be related to shear zones within the rock (see figure). Fluid inclusions within the anhydrous granulite patches are both CO₂-rich and isotopically distinct from those in the hydrous patches



Linear shear zones in a South Indian quarry along which mica-rich assemblages (pale rocks) have been dehydrated to form granulites (dark rocks). A classic example of dehydration from fluid infiltration.

of the quarry — a clear indication of a link between the dehydration process and the entrapped fluid⁶.

Geologists working on granulites from other areas, however, have not generally found such structures. Studies on granulites from the Adirondacks provide persuasive arguments (on the basis of both solid-phase equilibria and fluid-inclusion studies) for the absence of fluids during granulites facies metamorphism^{4,7}. Clearly granulites can form in different ways. The problem remains that although the south Indian studies have demonstrated that some granulites form by infiltration of CO₂, these were not lower crustal granulites but formed at intermediate depths of 15–20 km. Granulites formed at deeper levels may therefore result from different processes. Recent helium isotope studies have estimated the amounts of CO₂ entering the crust from the mantle and conclude that present-day CO₂ fluxes are too low to cause regional dehydration of the lower crust, at least in some actively tectonic areas⁸.

The argument over the abundance of CO₂ at deep crustal levels is now being eclipsed by a second argument concerning the effect it has on the minimum melting

temperature of common crustal mineral assemblages. Many granulites, particularly those formed at deep crustal levels, have been depleted in those elements which preferentially partition into a coexisting melt. It is therefore reasonable to assume that magma has been extracted from such depleted assemblages during granulite formation. Indeed, because water itself will partition into the melt, partial melting is often given as a possible mechanism for dehydration of hydrous minerals to form granulites.

But does an influxed CO₂ fluid lower the melting point of crustal rocks, as does water, or does it increase the melting point? Many geologists have interpreted the preliminary experimental results of Wendlandt⁹ to support the former proposition. This allows models of formation of granulites in the lower crust to incorporate both CO₂ influx and partial melting models because the fluid would trigger the melting. But at a meeting* last year, there was lively debate over the interpretation of these results and their application to the lower crust. The argument is important because if CO₂ increases the melting temperature of the crust, you can argue for CO₂ influx or for partial melting in the formation of granulites, but not both.

In the case of depleted high-pressure granulites, melting in the absence of fluid infiltration was favoured by many. In the latest twist to the story, reverse experimental results from Peterson and Newton in this issue² confirm that melting in some crustal rocks is promoted by CO₂. CO₂ influx is once again consistent with the observed depletion of magmaphile elements seen in deep-level granulites.

These results will not sway those who believe that CO₂ has no role to play in stabilizing lower crustal assemblages. The argument remains between those who believe that fluid-absent conditions, as seen in the granulites of the Adirondacks, are characteristic of conditions in the lower crust, and those who believe that CO₂ infiltration models, as exemplified by the quarries of south India, may better characterize lower-crustal processes. □

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1. Newton, R.C., Smith, J.V. & Windley, B.F. *Nature* **288**, 45–50 (1980).
2. Peterson, J.C. & Newton, R.C. *Nature* **340**, 378–380 (1989).
3. Touret, J. *Lithos* **4**, 423–436 (1971).
4. Lamb, W.M., Valley, J.W. & Brown, P.E. *Contr. Miner. Petrol.* **96**, 485–495 (1987).
5. Janardhan, A.S., Newton, R.C. & Smith, J.V. *Nature* **278**, 511–517 (1979).
6. Jackson, D.H., Matney, D.P. & Harris, N.B.W. *Nature* **333**, 167–170 (1988).
7. Lamb, W. & Valley, J.W. *Nature* **312**, 56–58 (1984).
8. O'Nions, R.K. & Oxburgh, E.R. *Earth planet. Sci. Lett.* **90**, 331–347 (1988).
9. Wendlandt, R.F. *Am. Miner.* **66**, 1164–1174 (1981).

* NATO Advanced Science Workshop on Granulites, Clermont Ferrand, 5–9 September 1988.

High performance

THE relationship between the sexes is very asymmetric. A woman can accept a lover with relatively little instinctive fervour — a fact long exploited by the oldest profession. A man, however, needs to demonstrate unequivocal biological interest. Often this is lacking; hence the centuries-old search for a true aphrodisiac. Now Daedalus has the answer.

He points out that the basic mechanism of male sexual excitement is relaxation. Erection requires relaxation of the muscle-sheaths of the arterioles which admit blood to the penis. Nerve poisons which relax such muscles can cause erection, as well as other more unpleasant symptoms.

Now botulinum toxin, one of the deadliest nerve poisons known, has recently been used to suppress muscle spasms. As a poison, it paralyzes its victims by inhibiting the release of the neurotransmitter that stimulates muscle contraction. But injected in an extremely small dose, it merely weakens the response of a muscle to nervous stimuli. In the muscles of the face, for example, it can abolish distressing tics and spasms without inhibiting normal facial expression. It degrades extremely slowly: a single injection can work for as long as four months.

So Daedalus is seeking volunteers for a study of botulinum toxin as a local aphrodisiac. A carefully measured locally injected dose should subtly weaken the contractile power of the penile arterioles. For four months afterwards, erection should be much easier to achieve and maintain, perhaps almost automatic; and the treatment can easily be repeated. Elderly gentlemen facing the decline of their virility, unhappy husbands fighting to maintain troubled marriages, Casanovas unnerved by scornful feminists, all would welcome this novel biochemical assistance. A great blow would be struck for sexual equality, for neither party to a liaison would now need to have much real interest.

But Daedalus goes further. He observes that other toxins, like dendrotoxin snake venom, have an opposite action to botulinum. They induce the steady release of neurotransmitter, thus driving the muscles into permanent contraction. So an injected dendrotoxin 'anti-aphrodisiac' should also be possible. It will reinforce the contraction of the penile arterioles and abolish erection for months at a time. Unlike the current drastically feminizing hormone treatments, this neat local sexual inhibitor will be perfectly controllable, completely reversible, and without psychological side effects. Eager customers for the new treatment will include monks seeking to defeat temptation, sexual offenders anxious to curb their criminal tendencies, and candidates for high political office in Japan and the United States.

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Valency and superconductors

SIR—Tranquada *et al.*¹ report X-ray absorption measurements on $\text{Nd}_{2-x}\text{Ce}_x\text{CuO}_4$, on the basis of which they claim that some Cu^{2+} ions are converted to Cu^+ ions by cerium doping and also that cerium is mixed-valent. In my opinion, these claims, which are relevant to theories of high- T_c superconductivity², should be reconsidered.

The technique used to determine valency involves the removal of a core electron. It is well known that, because of core-hole-induced changes in the final electronic state, the spectra may not reflect the true configuration of the initial state³. This issue has been discussed widely in the context of the valency of cerium in CeO_2 . We have shown^{4,5} that cerium is tetravalent in CeO_2 and that the mixed valency proposed by others is the result of covalent mixing of 4f levels in the valence band⁶. A similar situation might exist in $\text{Nd}_{2-x}\text{Ce}_x\text{CuO}_4$, in which case the X-ray absorption data alone would not be sufficient to establish the mixed valency of cerium. We suggest that cerium is in fact tetravalent in this superconductor also; but one must then ask how the Ce^{4+} state is possible in a metallic environment, in view of established ideas to the contrary^{7,8} in terms of kondo interactions.

The valency of Cu is a more serious issue. There are no major differences between the X-ray-absorption spectral features of copper in Nd_2CuO_4 and those in cerium-doped specimens. Those small differences in lineshape and linewidth that might exist may be ascribed to greater 3d hybridization in Ce-doped compounds and may be unrelated to a Cu^+ component; it is commonly believed that hybridization in the ground state may cause such effects.

Thus, current understanding of the processes involved in the X-ray absorption method, particularly for copper K-edge measurements, is not sufficient for any firm conclusions to be drawn regarding the valency of copper or cerium in these compounds on the basis of the data in ref. 1. Further experiments are required before the presence of Cu^+ or of mixed-valent cerium can be considered to be unambiguously established.

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TRANQUADA *ET AL.* REPLY—We agree with Sampathkumaran that further characterization of this material is required, but we stand by our previous analysis of the near-edge structures.

The changes observed at the copper K edge as a result of cerium doping are small on an absolute scale; they are quantitatively consistent, however, with the changes expected if one Cu^{2+} is converted

to Cu^+ for each cerium atom that replaces a neodymium. They cannot be explained by a simple shift of the energy scale. It would be helpful to have good cluster calculations of the X-ray absorption spectra for different initial-state configurations, as there are no good model compounds for the cerium-doped material. Nevertheless, we believe that the changes cannot be explained by the creation of the $3d^9 4s$ configuration, as some have suggested, but instead result from a mixing of $3d^{10}$ and $3d^9$ states. Furthermore, our interpretation is consistent with the expectation that, as in CuO , the first state to be filled on adding an electron should be the copper 3d hole⁹. Sampathkumaran suggests that the changes are due to hybridization effects. If doping causes changes in the hybridization, then similar effects should be seen in the hole-doped compound $\text{La}_{2-x}\text{Sr}_x\text{CuO}_4$, which we clearly do not see. The lattice parameters change by a very small amount with cerium doping¹⁰, and it is therefore unclear why one should expect any change in hybridization.

Measurements have now also been performed at the oxygen K and copper L_3 edges^{11,12}. The strong peak at the copper L_3 edge is found to decrease with cerium doping, indicating a decrease in the density of copper 3d holes. The sharp low-energy peak at the oxygen K edge probably corresponds to a transition from the oxygen 1s state to the copper 3d state, which is allowed as a result of oxygen 2p-copper 3d hybridization. The situation appears very similar to that found in NiO (ref. 13). The alternative interpretation given by the authors of ref. 12—that the oxygen K-edge measurements indicate the presence of holes of a predominantly oxygen 2p character—is difficult to reconcile with our copper K-edge results.

We do not really differ with Sampathkumaran as far as the valency of cerium is concerned. We agree that the cerium added to Nd_2CuO_4 is very similar to that in CeO_2 , and do not object to covalently-bonded cerium being called tetravalent. The important point is that the cerium donates extra electronic charge to the

system, and that charge appears to go into copper 3d holes.

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Not so fishy

SIR—John Maddox's comment¹ "Another red herring leads nowhere" on our recent paper² unfortunately contains some incorrect physics. Our work on angle-correlated two-photon lines in 6-MeV-per-nucleon collisions of $\text{U} + \text{Th}$, $\text{U} + \text{U}$ and $\text{Th} + \text{Th}$ provides evidence for approximately 180°-correlated two-photon lines up to about 1.1 MeV. Because the individual photons have approximately one-half of this energy, they cannot give rise to electron-positron pairs, as is implied in Maddox's comment. In fact, it had been shown several years ago that if the electron-positron pair lines found at the Gesellschaft für Schwerionenforschung, Darmstadt (GSI) were due to external or internal pair conversion, the corresponding single γ -rays would have to stand out significantly in any single photon spectrum. Such protruding γ -rays have not been found^{3,5}. Therefore, whatever the GSI electron-positron phenomenon may be, it is not simply "secondary products of single γ -rays released from nuclei put into high rotational states". At present, there does not seem to be any red herring, but simply unexplained scientific fact.

We wholeheartedly agree with Maddox that science sometimes needs to take risks and to use costly manpower and accelerators in order to progress. The results may not always be clear and their significance may take years of painstaking work to bear fruit. We are somewhat dismayed, though, to see the carefully done work on the GSI electron-positron phenomenon be put on a par with the initial studies on 'cold fusion'. In their style, the two studies appear not to belong in the same class.

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1. Tranquada, J.M., Heald, S.M., Moodenbaugh, A.R., Liang, G. & Croft, M. *Nature* **337**, 720–721 (1989).
2. Rice, T.M. *Nature* **337**, 686–687 (1989).
3. Sampathkumaran, E.V. *et al.* *Phys. Rev. Lett.* **54**, 1067–1070 (1985).
4. Kaindl, G. *et al.* *Phys. Rev. Lett.* **58**, 606–609 (1987).
5. Kaindl, G. *et al.* *Phys. Rev.* **B38**, 10174–10177 (1988).
6. Koelling, D.D. *et al.* *Solid State Commun.* **47**, 227–230 (1983).
7. Kalkowski, G. *et al.* *Phys. Rev.* **B32**, 2717–2720 (1985).
8. Allen, J.W. & Martin, R. *Phys. Rev. Lett.* **49**, 1106–1110 (1982).
9. Ghijssen, J. *et al.* *Phys. Rev.* **B38**, 11322–11330 (1988).
10. Takagi, H., Uchida, S. & Tokura, Y. *Phys. Rev. Lett.* **62**, 1197–1200 (1989).
11. Lin, C.L. *et al.* *Phys. Rev. B* (submitted).
12. Nücker, N. *et al.* *Z. Phys. B* (in the press).
13. Kuiper, P. *et al.* *Phys. Rev. Lett.* **62**, 221–229 (1989).

1. Maddox, J. *Nature* **339**, 253 (1989).
2. Danzmann, K. *et al.* *Phys. Rev. Lett.* **62**, 2353–2356 (1989).
3. Clemente, M. *et al.* *Phys. Lett.* **137B**, 41 (1984).
4. Bokemeyer, H. *GSI preprint No. 84/43* (1984).
5. Cowan, T. thesis, Yale Univ. (1988).

Species richness and the energy theory

SIR—I wish to point out some fundamental flaws in the data analysed by Turner *et al.* in their recent letter¹.

One difficulty with the study of the distribution of birds compared with other groups is that they are very highly mobile, and individual populations of the same species often react differently to changing seasons. The abundance and spread of several species also depends on population levels in the previous season, whether winter or summer. Several of the so-called resident species used in the analysis are by no means sedentary, and one, the bearded tit, is not insectivorous in winter. By definition, maps *a* and *c* in their Fig. 1 should at least show a similarity in distribution, or even a reversal of that shown, with many inland breeders moving coastwards for the winter.

The quoted winter surveys of 1981–84 were quantitative², and recorded a species present even if only one individual was observed during the three winters. These data can hardly be statistically valid in such an analysis. Four of the six winter visitors are very scarce, and would not be expected to show a representative distribution. Two (woodlark and black redstart) also breed, although the origin of the wintering birds is not clear. Furthermore, chiffchaffs do not show an urban winter distribution. Indeed, small insectivorous visitors to Britain in winter are neither widespread nor numerous. I also cannot understand the choice of habitats used in the analysis. It is not surprising that there is little correlation with species distribution. For the five types of natural habitat entered into the regression, nine of the resident species normally occupy none, and a further nine occupy only one.

Two other errors in the letter concern statements on cold weather and sunshine. First, the statement that cold weather kills birds is too simplistic. It is unusual for this to be a direct cause of death, and then only when temperatures persistently fall well below the normal variation found in a species' environment. In most cases a bird will survive cold weather as long as sufficient food remains available, and it is the sudden and marked reduction in food supplies due to hard frost, ice and snow-cover that causes widespread mortality, not the effect of low temperature itself.

Second, in a review on the responses of birds to sunshine and high temperatures³, I showed that feather conditioning is only one of several reactions. Sunning in birds is also thought to be undertaken for the manufacture of vitamin D, to enable ectoparasites to be removed more easily, and simply to warm the body. Thermoregulation may well be the more important function of sunbathing⁴.

In view of the points raised above, I remain sceptical of the authors' conclusions.

I am not convinced that there is any direct relationship between species richness and solar energy. This energy is, however, directly responsible for the distribution and abundance of vegetation and associated animal prey which provide nesting habitat and food for avian populations, that is, the habitat theory refuted by the authors, and that proposed by Wright⁵, who related species richness to total net primary production and not merely climatic variables.

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SIR—Recent suggestions^{1,5,6} that available energy, rather than other candidate causes⁷, predominantly explains latitudinal gradients of species richness need further clarification on the form such an energy theory might take. This can be illustrated by commenting on the recent paper of Turner *et al.*¹.

If the change in energy availability with latitude is the cause of the change in species number, then it is a prerequisite that such a change is mediated by change in the number of individual organisms. With less energy available, the numbers of individuals of all species might be reduced leading to zero members in some species. Members of different taxa are not equivalent in their energy demands¹. But important differences also occur within taxa, for example, between bird species of different sizes. The replacement of large bird species by small birds species could increase diversity under conditions of reduced energy input. Thus, any 'energy theory' of species diversity needs to take into account that it is the numbers of individuals of different body sizes that are

causally affected by any energy limitation. The relationship between the numbers of individuals of different sizes and the numbers of species is central to any energetics explanation of species diversity. Hutchinson⁸ suggested that latitudinal gradients in species number are caused by gradients in numbers of individuals which in turn are caused by gradients in solar energy input, the relationship possibly being mediated by body size⁹.

To test the relationship between body-size distributions of individuals and species in a community I examined 85 common bird census units (average 40 hectares (4×10^5 m²) each). The CBC¹⁰ records the abundance of all breeding bird species using species-specific techniques for mapping breeding populations. Of the 85 data sets, five had equal species and individual medians, 41 had species medians greater than individuals, for 39 the reverse was true; a χ^2 test ($v = 84$, $\chi^2 = 77.8$) indicated no evidence of difference between expected equal distribution of species and individual medians; Spearman's rank correlation for individual and species medians ($r = 0.70$ $P < 0.001$). If the median species weight and median individual weight in a community is closely correlated then this would establish an empirical link between diversity and energetics phenomena.

Body size is the subject of biogeographical study as Bergman's rule which can be applied to between and within species variation¹¹. Body size has also assumed an important role in explaining trophic structure, that is, how energy flows through a food web^{12,13}. The study of spatial distribution of body size therefore offers an integrating perspective to a biogeography based on energetic principles. Using data for British breeding birds¹⁴, the figure shows species with larger body size to be found at higher altitudes and

TABLE Correlations of species size and species abundance with environmental parameters

	Latitude	Altitude	January °C	July °C	Rainfall
All land species					
S per 2,500 km ²	-0.31 *	0.08 NS	0.07 NS	0.10 NS	0.10 NS
Mean weight	0.75 **	0.86 **	-0.75 **	-0.91 **	0.75 **
Median weight	0.69 **	0.74 **	-0.71 **	-0.81 **	0.57 **
Resident species					
S per 2,500 km ²	0.14 NS	0.49 **	-0.32 *	-0.36 **	0.39 **
Mean weight	0.75 **	0.87 **	-0.73 **	-0.91 **	0.76 **
Median weight	0.78 **	0.80 **	-0.75 **	-0.86 **	0.62 **
Summer migrant species					
S per 2,500 km ²	-0.74 **	-0.52 **	0.58 **	0.67 **	-0.38 **
Mean weight	-0.32 **	-0.17 NS	0.15 NS	0.24 NS	-0.05 NS
Median weight	-0.49 **	-0.29 *	0.34 **	0.42 **	-0.19 NS
Environmental variables					
Rainfall per year	-0.37 **	0.82 **	0.11 NS	-0.60 **	1.00
July °C	-0.91 **	-0.77 **	0.86 **	1.00	
January °C	-0.70 **	-0.28 *	1.00		
Altitude	0.69 **	1.00			
Latitude	1.00				

The number of species, S, found in each of the 90 areas shown in the figure, the mean and the median weight of the set of species in each area were correlated using Spearman's rank coefficient (* $P < 0.01$, ** $P < 0.001$) with environmental data from overlay maps¹⁴. January and July temperatures at sea level are adjusted by -0.6 °C per 100 m to surface temperature predicted from altitude overlay. NS, not significant.



Mean species weight of the British breeding bird land fauna. The presence or absence of the 158 breeding species of land bird recorded as breeding in each of 90 contiguous land areas of 2,500 km² covering Britain was determined from Sharrock¹⁴. Data are for 1968–73; each area may contain > 25 10 × 10-km squares if the latter extend beyond the coastline. The mean species weight is the set of species in each area, calculated using a single weight for each species (no intra-specific variation was examined). Data were interpolated and displayed using SYMAP¹⁵. The contour level is given by the number (1–4) at the centre of each area dataset. Small dots, 115.8–153.0 g; open circles, 153.0–191.3 g; circles with bar, 191.3–229.1 g; filled symbols, 229.1–266.9 g.

latitudes, see also the table. The summer migrants (predominantly insectivores) have correlations of different signs to the resident land avifauna, thus indicating that insectivores cannot be studied in isolation¹ if conclusions are to be generalized.

The study of latitudinal gradients of species richness has been strengthened by analysis from an energetics standpoint¹. But the 'energy theory' of species diversity is complex, less recent and embedded in trophic-structure arguments concerning body size.

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TURNER AND LENNON REPLY — As predicted by Elkins, the summer and winter distributions of resident birds are correlated ($r = 0.63$, compared with only $r = 0.47$ for the correlation between summer and winter visitors), but the 'surface' of species richness becomes 'flatter' in the winter. A slight alteration in the contour intervals used reveals the expected concentration

towards the coast. A comparison of correlations with short-period meteorological records with our correlations with the 30-year averages could be used to investigate the influence of recent population levels (and hence, recent weather) as against longer-term population histories on these seasonal distributions. The definition² of winter species richness does not differ materially from that used for the summer distribution¹⁴, where presence is recorded from a minimum of one pair giving adequate evidence of breeding.

The concentration of species to the south and along the coasts is shown also by our map of winter 'visitors'. Although the six species concerned are sparsely distributed, the survey¹ recorded over 3,300 individual sightings, and we doubt that the species richness is "unrepresentative" or "statistically invalid".

Elkins has confused various possible roles for 'habitat'. If this country is largely occupied by species which can coexist with British agriculture, forestry and townscape, then areas containing further 'special' habitats with their own specialized species might be expected to show a higher species diversity. The five habitats we tested favour such birds¹⁶, but the presence of any of them in a quadrat did not significantly increase its species richness.

An area containing a good diversity of habitat is likely to contain correspondingly more species. Although the general species poorness of the intensely agricultural Fenland is visible on the maps, our combined index of habitat diversity (which includes urbanization, which does increase diversity) likewise failed to explain species richness. This kind of habitat diversity is likely to contribute something to species richness, but an appropriate diversity index may be hard to devise.

Birds may indeed use direct infrared radiation for body warmth when sunbathing; but it is clearly a minor part of their energy budget, and we maintain that it is valid to contrast them with butterflies. Low temperatures do kill birds, by exhausting their fat-supplies to maintain body temperature, unless food is abundant — it would be interesting to see whether the inclusion of 'days of snow lying' in the statistics would improve the predictive value of winter temperature.

Cousins' findings provide a preliminary test of whether the effects of solar energy on species-diversity gradients are mediated through the overall productivity of the habitat or through the birds' energy budgets. Small birds have difficulty in maintaining body temperature in cold weather. That terrestrial birds become heavier (averaged across species) to the north seems to indicate that species with smaller body sizes become extinct more readily in cooler climates. But this applies only to resident species — summer visitors

show a much weaker increase in weight to the north, and no significant decline in mean weight with reduced temperature, being in the country only at a time of year when the temperature is generally high enough for them to need only slight increases in metabolic rate to cope with decreases in ambient temperature. Together with the findings of Root¹⁷, Cousins' results therefore suggest that the birds' own energy budgets are heavily involved in determining the diversity gradient during the winter, but that diversity gradients in summer are maintained largely independently of the birds' thermal budgets, most probably by the productivity of the environment.

Species with large body size must, for a given limited biomass, maintain smaller populations than smaller-sized species, and thus run more danger of stochastic extinction. If endotherms increase in weight to the north, this will further steepen the latitudinal gradient in their species richness. But the same effect should not be apparent for ectotherms. We suggest that a comparison between the gradients for butterflies and moths¹⁸ could test this hypothesis.

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1. Turner, J.R.G., Lennon, J.J. & Lawrenson, J.A. *Nature* **335**, 539–541 (1988).
2. Lack, P. *The Atlas of Wintering Birds in Britain and Ireland* (Poyser, Berkhamstead, 1986).
3. Elkins, N. *Weather and Bird Behaviour* 2nd edn (Poyser, Calton, 1988).
4. Simmons, K.E.L. *The Sunning Behaviour of Birds* (Bristol Ornithology Club, 1986).
5. Wright, D.H. *Oikos* **41**, 496–506 (1983).
6. Currie, D.J. & Paquin, V. *Nature* **329**, 326–327 (1987).
7. Begon, M., Harper, J.L. & Townsend, C.R. *Ecology* (Blackwell Scientific, Oxford, 1986).
8. Hutchinson, G.E. *Am. Nat.* **93**, 145–159 (1959).
9. Cousins, S.H. *Proc. 17th Int. Orn. Congr.* 1051–1055 (Deutsche ornithologen-gesellschaft, Berlin, 1980).
10. Williamson, K. *Bird Study* **11**, 1–22 (1964).
11. James, F.C. *Ecology* **51**, 365–390 (1970).
12. Cousins, S.H. *J. theor. Biol.* **82**, 607–618 (1980).
13. Warren, P.H. & Lawton, J.H. *Oecologia* **74**, 231–235 (1987).
14. Sharrock, J.T.R. *The Atlas of Breeding Birds of Britain and Ireland* (Poyser, Berkhamstead, 1976).
15. Dudnik, E.E. SYMAP (Univ. Illinois Press, 1968).
16. *The Atlas of Breeding Birds in Britain and Ireland. Transparent Overlays of Environmental Factors* (British Trust for Ornithology, Tring, 1976).
17. Root, T. *Ecology* **69**, 330–339 (1988).
18. Turner, J.R.G., Gatehouse, C.M. & Corey, C.A. *Oikos* **48**, 195–203 (1987).

Isomerase modification

SIR—With regard to the paper of Fischer *et al.*¹ on the unexpected identity of cyclophilin, a protein that binds cyclosporin A (CsA), and the enzyme peptidyl-prolyl *cis-trans* isomerase (PPIase), the following points may be of interest. First, the conclusion that one sulphhydryl group in PPIase is essential for enzyme activity is established by the identity of the PPIase modification and inactivation profiles²

rather than, as suggested by Fischer *et al.*, the effect of CsA on the kinetics of PPIase modification and inactivation. Second, the kinetic effects of CsA indicate that PPIase must be subject to processes of probable physiological significance such as protein-modification cooperativity, conformational isomerism and/or modification-induced protein unfolding^{3,4}.

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1. Fischer, G., Wittmann-Liebold, B., Lang, K., Kieffhaber, T. & Schmid, F. X. *Nature* **337**, 476–478 (1989).
2. Tsou, C.-L. *Sci. Sin.* **11**, 1535–1558 (1962).
3. Rakitzis, E. T. *J. math. Biol.* **10**, 79–87 (1980).
4. Rakitzis, E. T. *J. enz. inhib.* **1**, 289–299 (1987).

Fertility and cystic fibrosis

SIR—In a recently published case-control study¹, we demonstrated that cystic fibrosis (CF) carriers do not have higher fertility (measured by completed family size) than do matched controls. Unlike most previous studies, our analysis corrected for ascertainment bias. We also examined birth interval as an indicator of fecundity. A CF gene would gradually increase in frequency if carriers have shorter than average birth intervals (and thus shorter generation times), even in the absence of a fertility difference. Although there was a trend toward shorter birth intervals in CF carrier families, we concluded that this probably resulted from ascertainment bias. Pritchard² made the useful suggestion that birth intervals should be compared after matching cases and controls for completed family size. Here I report the results of such an analysis.

As in our previous study, I identified 143 carrier couples as grandparents of individuals affected with cystic fibrosis. The affected individuals were ascertained from clinical records of the Intermountain Cystic Fibrosis Center, Salt Lake City. Control couples were drawn from a computerized genealogical database containing 1.25 million members and matched on the basis of father's and mother's ages, date of marriage, and completed family size. Analysis was restricted to those case and control couples in which both members survived at least to age 50.

Twenty replicate sets of matched control couples (143 couples in each set) were drawn randomly from the database. The average interval between marriage and first birth among carrier couples (634 days) was less than that of controls in only 11 of 20 comparisons. Couples with intervals of less than 40 weeks were excluded from this comparison. The average between-birth interval among carriers (1,103 days) exceeded that of controls in 19 of 20 comparisons. These

results show that, after matching for completed family size, there is no evidence for greater fecundity in CF carriers than in controls.

Interpretation of these findings should be tempered with the caution that the present-day frequency of CF could be generated by a selective advantage of only 2 per cent¹. Such small differences in completed family size and birth intervals are fairly difficult to detect statistically³. Nonetheless, these results, along with the uneven prevalence of CF in different ethnic groups, argue against a reproductive advantage for CF carriers. The high incidence of CF in some European populations seems more likely to be due to genetic drift or disease resistance (see for example ref. 4).

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1. Jorde, L.B. & Lathrop, G.M. *Am. J. hum. Genet.* **42**, 808–815 (1988).
2. Pritchard, D.J. *Nature* **335**, 122 (1988).
3. MacCluer, J.W. *Ann. hum. Genet.* **42**, 59–75 (1978).
4. Baxter, P.S. *et al. Nature* **335**, 211 (1988).

Erythrocyte knobs and malaria

SIR—The recent report¹ of cytoadherence by erythrocytes infected with knobless *Plasmodium falciparum* needs to be set in the context of a large body of work originating in my laboratory. This work has clearly demonstrated that the numerous small protuberances on the surface of infected erythrocytes, which we called knobs, are important for the biology of falciparum malaria. It has also shown that specific proteins are exported by intracellular parasites and inserted at the membrane of the host cell, as summarized in ref. 2.

In *P. falciparum*, knobs appear on the surface of erythrocytes infected with asexual parasites about half-way through the 48-hour cycle of development. Their appearance is correlated with cytoadherence to endothelial cells and consequent sequestration in capillaries of certain organs, including the brain. This sequestration keeps the later-stage parasites away from the spleen, the chief organ for their destruction. It is also important in pathogenesis of the disease.

All falciparum parasites form knobs and are cytoadherent *in vivo*. Furthermore, cytoadherence is at the knobs, not at other parts of the erythrocyte. Under *in vitro* conditions or in splenectomized hosts, where there is no selective pressure by the spleen, knobs and cytoadherence may be lost independently either phenotypically or genotypically. Four phenotypes are possible with regard to these traits. K⁺C⁺ (both with knobs and

cytoadherent) is the only one known to occur naturally. K⁺C[−] and K[−]C[−] have been obtained from several isolates either *in vitro* or in splenectomized monkeys. The fourth, K[−]C⁺, described in ref. 1, was selected *in vitro* from a subline of the Palo Alto/Uganda strain isolated in 1966 (ref. 3) and since maintained in monkeys or in culture. Its cytoadherence has been demonstrated only *in vitro*; cytoadherence *in vivo* remains to be shown. If it is cytoadherent *in vivo* and can give a patent infection in intact *Aotus* monkeys, then such a K[−]C⁺ strain could exist in nature, unlike both the K⁺C[−] and K[−]C[−] strains.

Genotypically K[−]C[−] clones adapted to *Aotus* erythrocytes *in vitro* produce very light, barely detectable infections in intact *Aotus* monkeys⁴. Such monkeys, however, show resistance to challenge by an *Aotus*-adapted K⁺C⁺ wild-type strain. This is analogous to viral vaccination with a live attenuated strain. This promising approach could be developed by engineering a K[−]C[−] clone genetically unable to form gametocytes and hence not able to recombine in nature with a virulent strain.

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1. Udomsangpetch, R. *et al. Nature* **338**, 763–765 (1989).
2. Howard, R.J. *Prog. Allergy* **41**, 98–147 (1988).
3. Siddiqui, W.A. *et al. Am. J. trop. Med. Hyg.* **21**, 392–399 (1972).
4. Langreth, S.G. & Peterson, E. *Infect. Immunity* **47**, 760–766 (1985).

Mycoplasma control

SIR—The three antibiotics that Hay *et al.* report¹ using to eliminate mycoplasma from infected cell lines have been superseded by the considerably more effective 4-quinolone group of antibiotics, such as Ciprofloxacin.

In a recent study² of 26 cell lines treated with Ciprofloxacin we found no evidence of cytotoxicity, no treatment failures, and the elimination of the most prevalent *Mycoplasma* species, including *M. arginini*, *M. fermentans*, *M. hyorhinis* and *M. orale*. A 4-quinolone antibiotic is specifically marketed for the elimination of mycoplasmas from infected cell lines by Dainippon Pharmaceutical Co. Ltd.

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1. Hay, R.J. *et al. Nature* **339**, 487–488 (1989).
2. Mowles, J.M. *Cytotechnology* **1**, 355–358 (1988).

Dioxins in the dock

Alastair Hay

Agent Orange and Its Associated Dioxin: Assessment of a Controversy. Edited by A.L. Young and G.M. Reggiani. Elsevier: 1988. Pp.334. Dfl250.

SUMMING up the relationship between youth and experience, Mark Twain said that when he was in his early teens he thought his father was ignorant, that at eighteen the old man seemed to have learned a thing or two, and that by the time Twain was 21 it was astonishing how much the old codger knew. Applying the same scale to our present knowledge of Agent Orange, the defoliant the United States used to clear Vietnam's inland and coastal forests, we are aged about 17.

Few chemicals have acquired the notoriety attached to the contaminating dioxin that was found in Agent Orange in the late 1960s. A dioxin industry was spawned then and has since burgeoned. Seduced by its toxicity, shape and biological properties, scientists have delved ever deeper into cells to find out how this demon chemical acts on the message of life itself, DNA.

Dioxins (the plural is more appropriate, for there are 75 different types depending on their degree of chlorination and the position of the chlorines on the parent dioxin backbone) have also become the centre of regulatory action by government agencies. Dioxins are produced in many combustion processes and are now regarded as indicators of the efficiency, or otherwise, of incinerators. Dioxins will help determine whether these facilities combust waste or close down.

It was hardly surprising that the use of a dirty defoliant in an unpopular war in Vietnam should arouse controversy. Assessing the truth, or falsehood, of claims that Agent Orange and/or its dioxin have damaged people's health is what Alvin Young and Guiseppe Reggiani, the editors of this inelegantly titled book, set out to do. Both see their role as more that of historians than of players in the drama. Indeed, in one of the chapters Young writes that he has "not related the history of Agent Orange as a witness but as an outsider". As an Air Force officer involved in many government committees set up to assess the effects of the defoliant on the health of American veterans who served in Vietnam, Young was never just a witness. His tale, and that of some of his collaborators, is not mere observation either. Young's chronology of events, objective in the main, omits many items that would

provoke a different interpretation of some of the events he describes.

This is a partisan book, written by authors who all played a role in the dioxin-in-Agent-Orange drama and who have a commonality of view. Some had larger roles than others, and have deservedly been recognized for the contribution they have made. Reggiani is one of these. His account of the episode in which dioxin

although it is still too early to close the chapter on the cancer risk. For dioxins, it is their ability to cause cancer that causes concern.

Other sections of the book border on the offensive. One contributor, Barry O'Keefe, is a Queen's Counsel and member of the Australian Bar Association. O'Keefe was hired by Monsanto — a manufacturer of Agent Orange — to defend the company's product before the Australian Royal Commission investigating its effects on the health of Australian servicemen who were in Vietnam. In court, O'Keefe was rather like a brain surgeon wielding a scalpel to dissect the hypotheses and conclusions of the various scientists, and others, called to Australia to give evidence.

Here I must declare an interest, for I was on the receiving end of O'Keefe's questioning and I found the inquisition mighty challenging. Reviewing the advocate's chapter is a much easier task, and I regret that O'Keefe has here allowed his brief to extend beyond the courtroom doors, for his chapter is a rather undignified attempt at an exposé of the research of two Swedish epidemiologists. Some of their findings linking phenoxy-herbicide exposure with soft-tissue cancer may indeed be flawed, and O'Keefe did much to point this out in court. Sadly, however, he fails to apply his considerable skills to show that similar studies carried out by Monsanto epidemiologists were equally dubious. Moreover O'Keefe's attempts to 'rubbish' the Swedish findings lose some of their impact, for an editorial note points out that recent investigations in the United States confirm another of the Swedes' claims (that exposure to phenoxy herbicides, and to 2,4-D in particular, may increase the risk of non-Hodgkin's lymphoma).

Other and better books (such as Peter Schuck's *Agent Orange on Trial*, published by Harvard University Press in 1986) have been written about Agent Orange and dioxin. Young and Reggiani set out to assess, and perhaps resolve, some of the controversies about these chemicals, but they have failed. Belief that the defoliant damages lives has been fuelled, rather than dampened, by the \$180-million out-of-court settlement for US veterans of the Vietnam war who sued manufacturers of Agent Orange. Resolution of the controversy is not yet in sight. It will be some years before the fears about dioxins abate and they become just another family of chemicals. When they do, someone else can assess the controversy more fairly. □

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IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Before and after: untreated forest (top) 60 miles from Saigon, and (below) the devastating effect of spraying Agent Orange on the forest. The full effects of the defoliant on humans are not yet known, and many of the controversies surrounding Agent Orange remain unresolved.

ejected from a chemical reactor contaminated the Italian town of Seveso is one of the few restrained and objective chapters. This sad event, in which some 700 people were evacuated from the town, has had a great influence on governments everywhere and has changed preconceptions about the chemical industry. As medical director of Hoffmann-La Roche, the parent company of the Italian factory housing the runaway reactor, Reggiani was uniquely placed to influence events. Much to the chagrin of the Italian authorities at the time, it was Reggiani who insisted that the residents of Seveso were at risk, and should be evacuated. Events have since demonstrated that the local population has not suffered severe health problems,

Imperfect lunar voyage

P. Kenneth Seidelmann

The Motion of the Moon. By Alan Cook. Adam Hilger: 1988. Pp. 222. £35.

IN THIS book, the author provides a literary introduction to the history and basic concepts behind calculating the motion of the Moon. The methods are developed historically with verbal descriptions and a minimum number of equations; this approach has its advantages but also serious shortcomings.

The reader must recognize the book for what it is, and for its limitations. First, it is rather dated — other than citations of papers by the author, the most recent reference is 1983, and the general level of currency is about 1980. There are also general omissions and inconsistencies. Although there is a chapter on the applications of computers, the work of Babbage and Comrie is not mentioned. The relationship between the motion of the Moon and determinations of time, such as ephemeris time and the irregular rotation of the Earth, is omitted. As for the inconsistencies, the quoted accuracy of lunar laser ranging is between one-half metre

and ten millimetres; and on page 120 the planets are said to present no problems in determining the orbit of the Moon, while on page 161 their direct and indirect effects remain the most difficult part of the lunar theory. Those without a background in celestial mechanics or mechanics will be frustrated, I expect, by the name dropping which goes unaccompanied by explanations. Thus, the bases for classical equations are mentioned by name, but without any further discussion.

It is in Chapter 9, "The Applications of Computers", that the book's lack of currency is most apparent. Here, great emphasis is put on the use of computers for developing analytical theories. Numerical integrations are dismissed as being for short time periods, yet reference is made to a numerical integration covering 44 centuries. What we are not told is that the numerical integrations have been fitted to observations and that they are the source of the accurate ephemeris of today. The current analytical theories have been fitted to the numerical integrations; they have not been fitted to the observations.

The real shortcomings of analytical theories are that they do not converge predictably, and that to achieve the accuracies needed today requires more terms than can reasonably be determined and evaluated by current methods. The discussion of lunar librations does not mention the requirement that lunar librations must be integrated along with the lunar orbit because of the interrelationship between the two.

In the last two chapters, the author makes an attempt to bring things up to date. Unfortunately, he is not successful. Constants are quoted at random, without an explanation of the sources. The requirement to know where the Earth station is, along with the lunar station, when performing lunar laser ranging, is forgotten. And although Cook says that no calculation of the lunar orbit that incorporates relativity has been carried out, he himself cites a paper by Newhall *et al.* that does just that. The requirements of relativity for time, light-bending and equations of motion are not mentioned anywhere in the volume.

If supplemented by material on analytical developments and on recent work, *The Motion of the Moon* will serve as an introductory textbook for courses on lunar theory. But it is not an accurate or up-to-date account of the subject. □

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New in Britain

• *The Stone-Age Health Programme: Diet and Exercise as Nature Intended.* by S. Boyd-Eaton, M. Shostak and M. Konner. (Angus & Robertson, £4.95). For review see *Nature* 336, 282; (1988).

Arm waving

Edwin W. Taylor

Cell Movement. Edited by Fred D. Warner *et al.* Alan R. Liss: 1989. Vol. 1 Pp. 356; Vol. 2 Pp. 494; Vol. 1 \$88, £78.50; Vol. 2 \$98, £87.50.

CILIA and flagella are extremely complex structures compared with muscle myofibrils, possibly because the propagation of a bending wave is more difficult to achieve than the longitudinal sliding of muscle filaments that ultimately produces force. In 1965, Gibbons and Rowe showed that the arms of the nine 'outer-doublet' microtubules in cilia (see figure) consist of a high-molecular-mass ATPase which they named dynein. By analogy with muscle contraction, the authors suggested that doublet microtubules and dynein form a type of motile system in which the dynein arms are equivalent to myosin crossbridges.

Slow but steady progress was made over the next 15 years by the handful of investigators who were willing to grapple with this difficult system. Was the postulated complex structure really necessary for a microtubule-based motile system? Could cytoplasmic microtubules and dynein produce linear movement rather than bending waves, and could such a system be responsible for particle movements within cells, such as chromosome movement during mitosis? Although dynein-like proteins were discovered in the cytoplasm, it was argued that they could be precursors of cilia or soluble proteins which became bound to the mitotic apparatus during its isolation.

New methods developed during the past six years have revived interest in old questions. The refinement of light-microscope techniques, in particular by R.D. Allen, allowed single microtubules to be observed. *In vitro* assays for motile systems developed by Spudich and Sheetz for actomyosin were applied to microtubule systems. But it turned out to be vesicle transport in neurons which provided the key to the problem. Particles had been observed moving along axons in both directions at velocities of the order of 1 µm per second. These velocities are comparable to filament-sliding velocities in muscles. In 1985, a previously unknown translocator protein, kinesin, was purified from squid axoplasm — this protein could account for anterograde transport, as vesicles are moved by kinesin towards the 'plus', or growing, end of microtubules. A similar protein was obtained from sea urchin eggs and subsequently from various cells and tissues. Another protein in axoplasm moves particles in the retrograde direction *in vitro*. This protein, then known as microtubule-associated protein-

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1C, was first purified from sea urchin eggs and mammalian brain, and turned out to be the cytoplasmic dynein.

Microtubule-based motility immediately became an exciting field of cell biology. Reports on kinesins and cytoplasmic dyneins began to appear from several laboratories in quick succession. The rapid expansion of this field is now described in the two-volume work entitled *Cell Movement*, to which most of the main figures in the field have contributed chapters. Volume 1 deals with cilia and flagella dyneins, the structure of cilia and flagella, and the mechanism of their movements. Volume 2 covers kinesin, cytoplasmic dynein, structure and dynamics of microtubules, particle movements and mitosis. Each section is introduced by a chapter providing a perspective on the topic.

Several articles concern the dynein ATPases of cilia and cytoplasm, which constitute a family of closely related proteins differing in the size and number of heavy chains in each molecule, and in the composition of the smaller subunits. The microtubule-activated ATPase mechanism has been studied in detail for the outer-arm dynein of the protozoan *Tetrahymena* by Johnson and collaborators, and the

development in muscle. The two or three heads of dyneins are different and structural and biochemical information (reviewed by Mitchell and Warner) could mean that head interactions are a part of the mechanism in dynein-mediated movements.

It is somewhat surprising that kinesin, a much smaller and apparently simpler protein, is able to do the same trick as dynein, although in the opposite direction. The gene has been cloned and its sequence determined by Goldstein, who contributes an interesting chapter on what molecular genetics can reveal about motile systems. Because the globular head portion of kinesin is only half as large as its muscle equivalent, myosin subfragment-1, it could be the simplest mechanochemical transducer yet discovered. Its biochemical properties are summarized in the chapter by Scholey *et al.* Very little is known about the kinetic mechanisms of kinesin except for the observations of Hackey that ADP dissociation is the rate-limiting step for kinesin ATPase and that the rate constant is increased by microtubule binding. Some variant of the actomyosin-type schemes developed for muscle could therefore apply to kinesin. The four chapters on kinesins provide a fairly

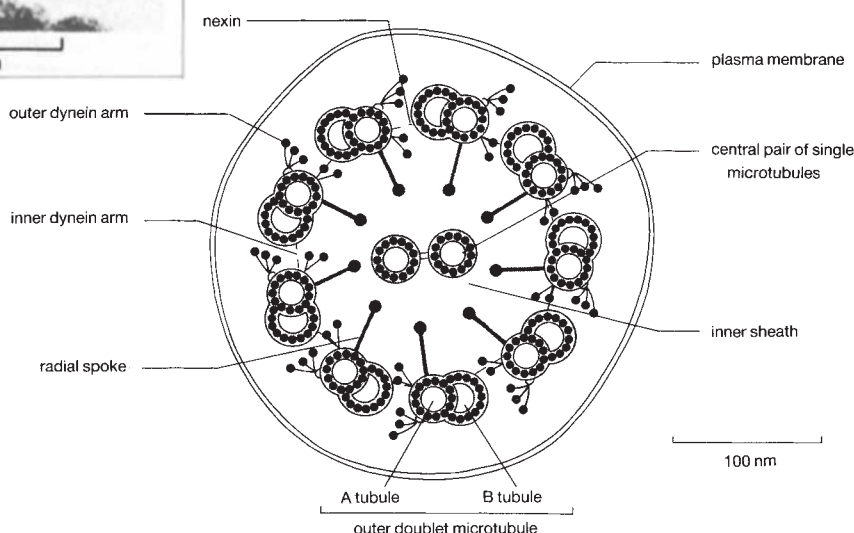
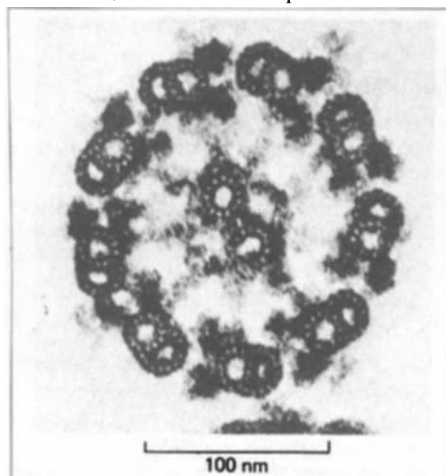
complete survey of what is known about this protein. Apart from its function in moving particles in neurons, very little is known about kinesin's role in other cell types.

The final section of volume 2 brings us up to date on a problem which has fascinated biologists for a

hundred years — microtubule movements during mitosis. Mitosis is a subject that had almost drowned on a sea of useless speculations until a new generation of investigators came to the rescue. Although the subject may still be treading water, real progress has been made by breaking the problem down into the chief steps and devising experiments to address each one. The section on mitosis and the chapters on microtubule structure and turnover provide a flavour of the recent advances in this field. The dynamic instability model of Mitchison and Kirschner provides a plausible explanation for how chromosomes attach to the mitotic spindle. An old controversy of whether chromosomes moved to the pole by depolymerization of microtubules or by a translocator ATPase, has been reformulated in modern terms. The mechanisms of formation of the metaphase plate and the contribution of active sliding of microtubules to spindle elongation in anaphase are presented in chapters by Mitchison, Salmon, McIntosh and Cande *et al.* The main questions may not have been answered, but critical experiments have led to significant progress in defining the problems.

These volumes do not constitute a textbook in which the field of microtubule-based motility is laid out in a logical manner, but are more in the nature of a progress report contributed by most of the leading investigators in the field. The authors may not agree with each other but the articles convey the sense of excitement that has been generated by the remarkable progress made during the past five years. □

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Ciliary movement: the main picture shows a cross-sectional diagram of a cilium; inset, an electron micrograph of a green-algal-cell flagellum, again in cross-section. Both pictures are taken from *Molecular Biology of the Cell*, published by Garland.

by a mechanism which is not clearly understood. Changes in the structure of the dynein arms during the force-generating cycle are discussed in *Cell Movement* by Goodenough and Heuser and by Satir. Although considerable progress has been made, we seem to be a long way from understanding the molecular mechanism of mechanochemical coupling. This problem has not been solved for muscle contraction even though we know much more about the structure and properties of actomyosin.

The several chapters on dyneins provide considerable information but little understanding of the puzzling observations — the complexity of dyneins and the presence of different kinds in the inner and outer arms of cilia. Interactions between myosin heads do not appear to be important for motion or force

The energetic British

Peter C. Hinkle

Bacterial Energy Transduction. Edited by Christopher Anthony. Academic: 1988. Pp. 517. \$98, £47.

BACTERIA have found a remarkable number of ways to make a living. Indeed, the information about alternative pathways for oxidative, photosynthetic and transport reactions is so vast that few try to master it all.

To facilitate teaching in this area nine British biochemists have contributed to this advanced text, and have succeeded in providing both introductory parts and well-coordinated reviews that will be very useful, both for students and researchers. The emphasis is on electron-transfer chains. There are two chapters on the relatively new field of periplasmic electron transport, as well as thorough coverage of anaerobic, chemolithotrophic, oxidative and photosynthetic electron transport.

The work is dedicated to Peter Mitchell, and the mechanisms of energy transduction are analysed in terms of Mitchell's chemiosmotic theory. The last chapter, by a chemiosmotic heretic, tries to undermine the general acceptance of that theory, but does not succeed. Presumably, the editor thought it would be healthy for students to be exposed to what remains of the once strong opposition to chemiosmosis, because he instructed the author to end with the statement that "we should not believe all that we read (including this chapter)".

The main weakness of the book is the limitation to British authors, which has caused some important topics to be left out and others to be covered with less enthusiasm than original researchers would have provided. There is no coverage of chemotaxis and the flagellar rotor, an advanced field that is certainly one of the most interesting aspects of bacterial energy transduction. The recent determination of the crystal structure of photosynthetic reaction centres is reviewed in its relation to electron transfer, but the actual structure of the protein is not presented. Bacteriorhodopsin is barely mentioned. The extensive sequence information on bacterial transport proteins is poorly covered and the techniques of molecular biology, which are central to recent advances in the field, are largely ignored. Perhaps those of us in the rest of the world should consider writing a second, companion volume to fill in the gaps. □

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At the bottom of a lake — early Holocene lake sediments from Taoudenni in the central Sahara, subsequently eroded by the wind to form pillars. The picture is taken from *The Holocene: An Environmental History* by Neil Roberts, published by Basil Blackwell.

Frameworking

L. V. C. Rees

Molecular Sieves: Principles of Synthesis and Identification. By R. Szostak. Van Nostrand Reinhold: 1989. Pp. 524. £53.95, \$75.95.

An Introduction to Zeolite Molecular Sieves. By Alan Dyer. Wiley: 1988. Pp. 149. £29.50, \$64.95.

ALTHOUGH synthesis of zeolites was an important component in the growth of research on these materials during the 1960s and 1970s, it was not until 1982, and the appearance of Barrer's *Hydrothermal Chemistry of Zeolites*, that the topic was covered by a monograph. The book dealt with many different aspects of the synthesis reaction from a more academic point of view, and helped greatly in putting the subject onto a firm scientific footing. Five years later, Jacobs and Martens published *Synthesis of High-Silica Aluminosilicate Zeolites*, a timely volume which contained a number of selected, proven recipes for high-silica zeolites, as well as detailed discussions of the synthesis of a number of different groups of such zeolites.

Now we have Rosemarie Szostak's *Molecular Sieves*. Szostak deals with the basic principles of synthesis, as did Barrer, but she puts greater emphasis on the practical aspects of the subject and is more concerned with methods of characterizing

the products. Also included is a large section on non-aluminosilicate molecular sieves, which contains many examples of zeolites where the framework aluminium has been partly or completely replaced by a number of other elements. This section, which complements the material covered in Jacobs and Martens, also contains a broad and important survey of aluminophosphate, silicoaluminophosphate and metalloaluminophosphate molecular sieves.

I received *Molecular Sieves* just before departing on a three-week visit to the National Chemical Laboratory in Puna, India. The laboratory has a large group working on the synthesis of many of the materials discussed by Szostak. The value of her book is exemplified by the fact that it was in continuous demand by all members of the group — I saw little of it during my stay.

Alan Dyer, author of the second volume reviewed here, has been involved with zeolites for over 30 years, and is thus well qualified on the subject. His is an introductory account, and in the space of only 145 pages he describes clearly most aspects of the properties and uses of zeolites, both natural and synthetic. This book will be essential reading for any undergraduate encountering zeolites and molecular sieves for the first time, and for chemists who wish to acquire a basic knowledge of these materials. □

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Disequilibrium and macrosegregation during solidification of a binary melt

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The crystallization of a liquid mixture in the presence of natural convection is an important problem in geophysics and metallurgy. Simple aqueous solutions may be used as analogue systems to study this process. Both theory and experiment reveal that convective motions in the fluid are important in determining both the rate of solidification and the development of compositional stratification in the solid. Moreover, convection may result in solidification in regions other than near the boundary at which cooling is imposed.

WHEN a melt of two or more components solidifies, the composition of the resulting solid is generally different from that of the melt. For example, salt water in the oceans freezes to form almost pure ice, and the partial solidification of a melt containing comparable quantities of germanium and gallium produces almost pure germanium. This compositional difference between the solid and melt indicates that the composition of the fluid in the near vicinity of the crystallization front differs from that in the bulk of the fluid, which generally implies that the density is also different. Contrasts in density can drive intense convective flows. Fluid mechanical effects should thus play a large role in solidifying systems, whether the final products are small-scale semiconductor crystals or the solid inner core of the Earth.

In the simple but fundamental situation of the cooling of an initially homogeneous melt at a single horizontal boundary, six different flow regimes have been identified¹ which depend on whether the thermal field results from cooling at either an upper or lower boundary to the melt and whether the fluid released on solidification has a greater, a lesser, or the same density as the melt. Building on previous investigations², we concentrate here on cooling, at an upper horizontal boundary, a melt that releases less-dense fluid on solidification. We highlight some novel interactions between fluid convection and crystallization. Identifying each new effect in turn, we present mathematical models to quantify the outcome in each case and describe its significance. Our theoretical predictions compare well with results from our laboratory experiments in which we use various aqueous solutions such as isopropanol and sodium sulphate. These are easy to handle in the laboratory and mimic the behaviour of a wide variety of binary alloys with which it is much more difficult to conduct controlled experiments.

The theoretical development incorporates the analysis of regions where solid dendritic crystals and melt coexist as a 'mushy' region^{3,4}. Figure 1 shows photographs of a mushy region consisting of solid ice and isopropanol-enriched water. Our aim is to calculate the rate of growth of the mushy layer and the variation in the fraction of solid ice within it. We first consider the mushy layer to grow under conditions of thermodynamic equilibrium. This leads to results in good, but not perfect, agreement with our experimental observations. The agreement is improved by permitting the melt to depart from thermodynamic equilibrium and to become supersaturated. In addition, this leads to an explanation of solidification at the floor, which occurs even though the system is cooled at the roof. Only if the temperature at the roof is less than the eutectic temperature of

the melt can complete solidification take place. In this case, compositional zonation of the solid is observed. This is a consequence of crystallization at the floor, which releases solvent into the melt and results in an evolution of the composition of the melt.

The model system and equations

Figure 2a illustrates the model system that forms the basis for our study. A rectangular container is filled with a two-component melt (or solution) of initially uniform composition C_0 and temperature T_0 . At the start ($t=0$) the upper boundary is instantaneously cooled to, and subsequently maintained at, a temperature T_b that is lower than the liquidus temperature $T_L(C_0)$ of the solution. All the other boundaries are considered to be thermally insulated.

In our first investigations T_b was greater than the eutectic temperature T_e of the binary system. The melt could therefore

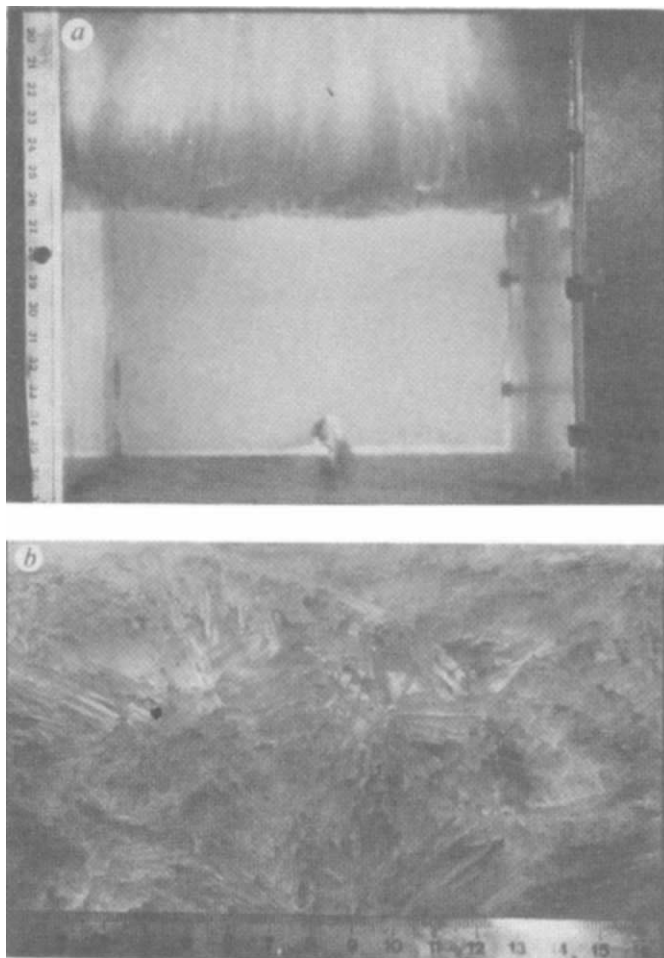


FIG. 1 Photographs of the mushy layer in an isopropanol experiment. *a*, A side view showing that the interface with the melt is approximately flat. *b*, A close-up view normal to the interface, showing the closely-spaced, plate-like ice crystals. This fine-scale structure allows the mushy layer to be treated theoretically as a continuum.

never be completely solidified. The behaviour that occurs if the fluid released on solidification is less dense than the melt is illustrated by our laboratory experiments with mixtures of isopropanol and water. Ice formed at the upper boundary and grew downwards in thin plates to form the mushy layer depicted in Fig. 1. The fluid in the interstices of the mushy layer was enriched in isopropanol as a result of the selective freezing of water to form ice from the binary melt. The enriched isopropanol solution was less dense than the original solution and remained stagnant within the mushy layer. Below the mushy layer we observed turbulent thermal convection of the fluid in response to the cooling from above. This convection played an important role in the subsequent evolution of the system. The experiments were continued until the lower edge of the mushy layer had descended to within a centimetre or so from the base of the tank, which took ~ 25 hours. The position of the mush-liquid interface and the temperature of the well mixed liquid region were monitored at intervals throughout the experiments.

To evaluate the experimental results and apply our findings generally to other systems, we have developed a mathematical model based on our understanding of the physical processes involved in the laboratory experiments. Equations governing heat and mass conservation within the mushy layer are derived from ref. 5. Transport of heat is governed by thermal diffusion and is described by

$$c_m \frac{\partial T}{\partial t} = \frac{\partial}{\partial z} \left(k_m \frac{\partial T}{\partial z} \right) + \mathcal{L}_\beta \frac{\partial \phi}{\partial t} \quad (1)$$

where T is the local temperature and ϕ the local volume fraction of solid dendrites. Changes in ϕ result in an internal release of latent heat which must be conducted through the mushy layer. The thermodynamic parameters are the specific heat per unit volume c , the thermal conductivity k and the latent heat per unit volume of solid $\mathcal{L}$. Subscripts β, m and later l denote properties of the solid phase forming the dendrites, the mushy layer and the liquid respectively. We evaluate the thermal properties of multi-phase media by equating them to local averages, weighted by the volume fraction, of the properties of the constituent phases. So, for example, the specific heat per unit volume and the thermal conductivity of the mushy layer are given by

$$c_m = \phi c_\beta + (1 - \phi) c_l \quad (2)$$

and

$$k_m = \phi k_\beta + (1 - \phi) k_l \quad (3)$$

Whereas equation (2) is exact, equation (3) is only approximate because conductivity depends on the microscopic morphology. However, equation (3) is an exact expression for a laminated material when the thermal gradient is aligned with the laminae⁶, and it has led to excellent results in previous analyses of stagnant mushy layers^{1,5}.

Huppert and Worster¹, and Worster⁵ demonstrate that the depth of a stagnant mushy layer is determined principally by thermal balances. Therefore, as the thermal diffusivity is typically much greater than the solutal diffusivity, we assume that vertical diffusion of solute is negligible. Conservation of solute in the mushy layer can then be expressed by a differential form of the Scheil equation³,

$$(1 - \phi) \frac{\partial C}{\partial t} = (C - C_\beta) \frac{\partial \phi}{\partial t} \quad (4)$$

where C is the concentration of the interstitial liquid and C_β is the uniform concentration of the solid dendrites. Equation (4) is readily integrated to show that the bulk, horizontally averaged composition $C_H = (1 - \phi)(C - C_\beta)$ is a function of z only. Finally, we couple equations (1) and (4) by using the linearized liquidus relationship.

$$T = T_L(C) \equiv T_L(C_0) + \Gamma(C - C_0) \quad (5)$$

where Γ is a constant, on the assumption that the mushy layer is in local thermodynamic equilibrium.

Conservation of heat across the thermal boundary layer at the moving interface between mush and liquid requires that

$$[c_l(T_l - T_i) + \mathcal{L}_\beta \phi] \dot{h}_i = k_m \frac{\partial T}{\partial z} \bigg|_{z=h_i} - F_T \quad (6)$$

where $h_i(t)$ is the position of the interface, T_i is the temperature there and F_T is the convective heat flux from the liquid to the mushy region. Conservation of heat in the region of well mixed liquid is expressed by

$$c_l(H - h_i) \dot{T}_i = -F_T \quad (7)$$

where H is the initial depth of the liquid. The heat flux F_T is approximated by the well-known semi-empirical relationship for turbulent convective heat transfer across a horizontal surface

$$F_T = (2^{4/3} \lambda) k_l \left(\frac{\alpha g}{\kappa_l \nu} \right)^{1/3} (T_l - T_i)^{4/3} \quad (8)$$

where g is the acceleration due to gravity, α is the coefficient of thermal expansion of the liquid, κ_l is its thermal diffusivity, ν is its kinematic viscosity and λ is an empirical constant. This relationship, which is often expressed in the dimensionless form $Nu \propto Ra^{1/3}$, where Nu is the Nusselt number and Ra is the Rayleigh number, can be derived from the assumption that the turbulent heat flux is independent of the depth of the convecting region⁷.

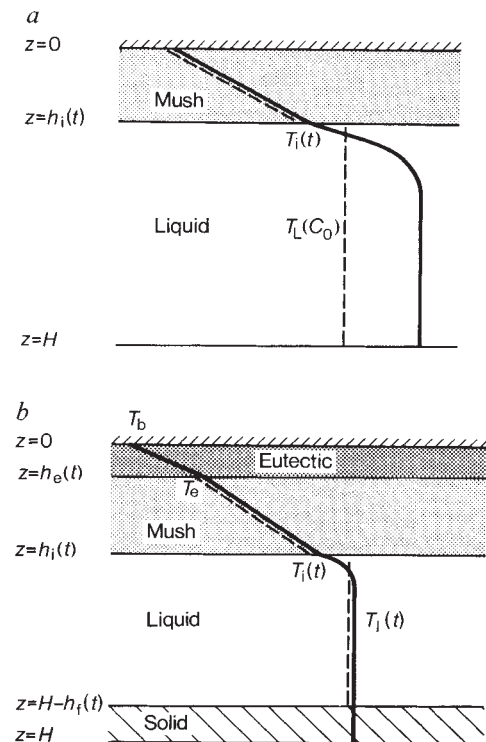


FIG. 2 Definition sketches of two models (*a, b*) for the growth of a mushy layer below a cold horizontal boundary maintained at a fixed temperature T_b . Throughout the mushy layer, the temperature (solid line) is constrained to equal the liquidus temperature (dashed line) of the interstitial melt. The interfacial temperature T_i is less than the equilibrium freezing temperature of the melt $T_L(C_0)$. Turbulent convection of the melt keeps its temperature uniform. Diagram *a* corresponds to our experiments with isopropanol. The boundary temperature is greater than the eutectic temperature T_e and a mushy layer forms adjacent to the cooled boundary only. In *b* there are two additional layers. The first is a layer of composite solid that grows on the roof when $T_b < T_e$. The second, which usually appears once the melt temperature T_i reaches $T_L(C_0)$, is a solid layer of crystals that grows on the base at a rate sufficient to keep the melt on the liquidus. The temperature within the basal solid is taken to be equal to T_i .

To complete the model we adopt the condition of marginal equilibrium⁵, which states that the temperature gradient in the boundary layer in the liquid ahead of the mush-liquid interface is equal to the gradient of the local liquidus temperature. This condition, combined with conservation of solute at the mush-liquid interface, leads to

$$\phi = 0, \quad C = C_0 \quad (\text{at } z = h_i) \quad (9a, b)$$

in the limit of zero solute diffusivity. We note that equations (9b) and (5) imply that

$$T_i = T_L(C_0) \quad (10)$$

whereas equations (9b), (4) and its integral imply that the bulk, horizontally averaged, composition C_H throughout the mushy layer is equal to the initial concentration C_0 . The solid fraction is thus given by

$$\phi = \frac{C_0 - C}{C_\beta - C} \quad (11)$$

It is instructive and useful to note that equations (1)–(5) can be combined into the nonlinear diffusion equation

$$\left[c_m + \frac{\mathcal{L}_\beta}{\Gamma} \frac{1 - \phi}{C_\beta - C} \right] \frac{\partial T}{\partial t} = \frac{\partial}{\partial z} \left(k_m \frac{\partial T}{\partial z} \right) \quad (12)$$

which shows that the release of latent heat acts simply to increase the thermal inertia of the mushy layer.

This system of equations was integrated numerically to determine the evolution of h_i and T_i . Figure 3 shows the results which are compared with data from the experiments with isopropanol. Very good agreement is found between theory and experiment for the growth of the mushy layer (Fig. 3a) and the cooling rate of the solution (Fig. 3b). The data show, however, that the temperature of the solution fell below the liquidus temperature during the course of each experiment. Such supersaturation, which cannot be accounted for with an equilibrium model, can

have extremely important consequences for the evolution of the system, as we demonstrate below.

Disequilibrium

Crystal growth is necessarily a non-equilibrium process; some level of supersaturation must exist in the vicinity of a growing crystal interface to drive solidification. Usually the departures from equilibrium are small and good predictions of growth rates can be made by assuming that the system evolves through equilibrium states, as we have just demonstrated. A more accurate formulation of crystal growth takes account of the interfacial kinetics involved by recognizing that the rate of growth is a function of the local supersaturation^{8,9}. We replace the equilibrium assumption leading to equation (10) by the simple linear relationship

$$\dot{h}_i = \mathcal{G}(T_L - T_i) \quad (13)$$

where T_L is the liquidus temperature at the interface and $\mathcal{G}$ is a constant. The interface temperature is now less than T_L and $C_i < C_0$. Correspondingly, as there is still no solute flux across the interface, the solid fraction at the interface, given by equation (11) with $C = C_i$, is greater than zero⁹. By measuring carefully during many experiments the evolution of the depth of the mushy layer $h_i(t)$ and the temperature at the interface T_i , using a 1-mm bead thermistor, we have confirmed equation (13) for the water-isopropanol system for supersaturations $T_L - T_i$ up to 3 °C and measured $\mathcal{G}$ as $\sim 2.2 \times 10^{-4} \text{ cm s}^{-1} \text{ °C}^{-1}$.

The kinetic growth law, equation (13), gives a timescale $\tau_g = H/\mathcal{G}(T_L - T_b)$ associated with disequilibrium that can be compared with the timescale for thermal diffusion, $\tau_\kappa = H^2/\kappa$. The ratio $\varepsilon = \tau_g/\tau_\kappa$ is typically very small; for example, it is 1.2×10^{-2} in our experiments with isopropanol. This indicates that when diffusion is the only transport process^{1,5}, departures from equilibrium are negligible except at very early times, of order $\tau_g = \varepsilon \tau_\kappa$, compared with the solidification time, which is of order τ_κ . We

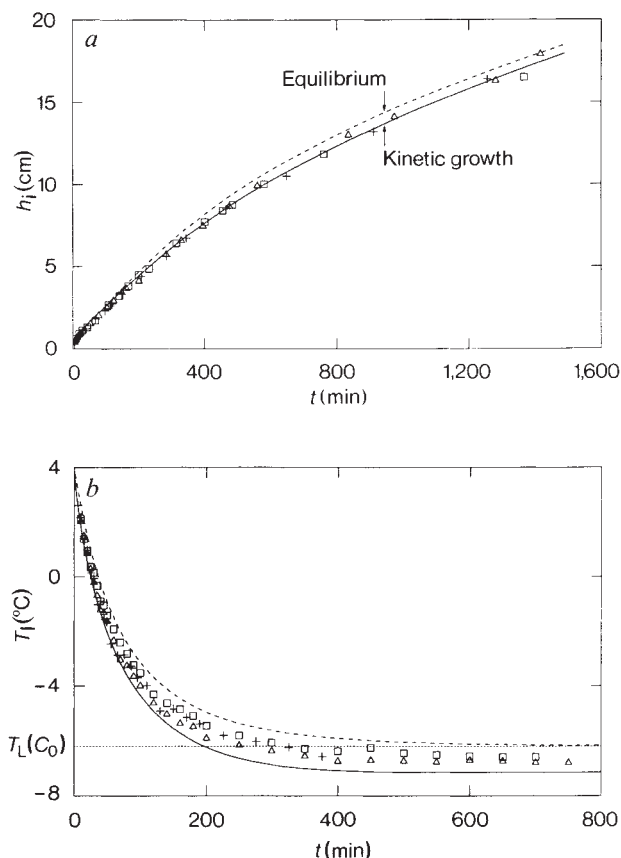


FIG. 3 a, The depth of the mushy layer h_i ; b, the temperature of the solution T_i versus time including data obtained from isopropanol experiments in which $T_b = -25$ °C, $C_0 = 83.2$ wt% H₂O and $T_0 = 4.0$ °C. The height of the tank $H = 18.8$ cm. The dashed upper curve was calculated by using the equilibrium assumption that the interface temperature T_i is equal to the initial liquidus temperature of the solution $T_L(C_0)$. The solid lower curve was calculated by using the kinetic growth law, equation (13), to determine T_i , and gives a better fit to the data. The horizontal dotted line in b indicates the liquidus temperature of the solution (-6.2 °C). In the calculations $c_\beta = 1.832 \text{ J cm}^{-3} \text{ °C}^{-1}$, $c_l = 3.912 \text{ J cm}^{-3} \text{ °C}^{-1}$, $k_\beta = 0.022 \text{ W cm}^{-1} \text{ °C}^{-1}$, $k_l = 0.0037 \text{ W cm}^{-1} \text{ °C}^{-1}$, $\mathcal{L}_\beta = 306 \text{ J cm}^{-3}$, $\nu = 0.057 \text{ cm}^2 \text{ s}^{-1}$, $\lambda = 0.056$ and the liquidus was represented by $T_L = -6.2 + 0.65(C - 83.2)$, where C is in wt% H₂O and T_L is in °C. All these values were determined from published data^{20–25}. Our own measurements of α suggested $\alpha = (2.25 + 0.157) \times 10^{-4} \text{ °C}^{-1}$, where T , in °C, was taken to be the mean of T_i and T_L .

shall see, however, that these small departures are important when coupled with convection of the melt.

The non-equilibrium model obtained by employing equation (13) rather than equation (9) is seen in Fig. 3a to give excellent agreement with experimental data for the growth of the ice. It also accounts for the occurrence of supersaturation in the liquid region after ~ 200 min. Note, however, that, owing to heat gain from the laboratory, there is a slight discrepancy between the predicted and experimentally observed results for the level of supersaturation.

Secondary crystallization

The experiments with mixtures of water and isopropanol were metastable for much of their evolution. That is, had there been sites for nucleation within the supersaturated liquid region then crystals could have grown anywhere in that region in addition to the crystals growing within the mushy layer. Such secondary crystallization has been observed, both by us and by previous authors², in laboratory experiments with various aqueous solutions. Figure 4 shows photographs of one of our experiments using sodium sulphate. In all these cases the solid forming the

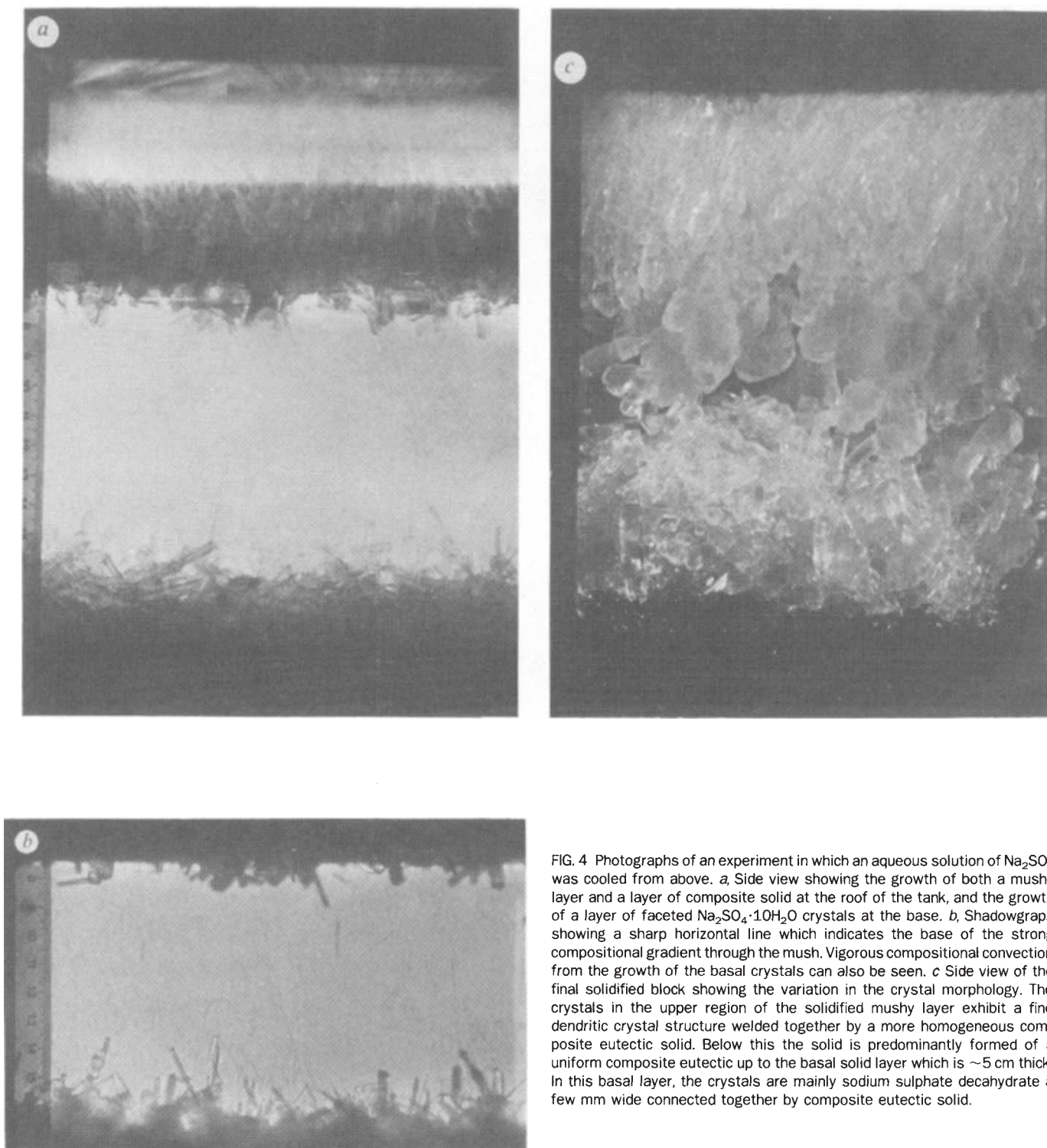


FIG. 4 Photographs of an experiment in which an aqueous solution of Na_2SO_4 was cooled from above. *a*, Side view showing the growth of both a mushy layer and a layer of composite solid at the roof of the tank, and the growth of a layer of faceted $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ crystals at the base. *b*, Shadowgraph showing a sharp horizontal line which indicates the base of the strong compositional gradient through the mush. Vigorous compositional convection from the growth of the basal crystals can also be seen. *c* Side view of the final solidified block showing the variation in the crystal morphology. The crystals in the upper region of the solidified mushy layer exhibit a fine dendritic crystal structure welded together by a more homogeneous composite eutectic solid. Below this the solid is predominantly formed of a uniform composite eutectic up to the basal solid layer which is ~ 5 cm thick. In this basal layer, the crystals are mainly sodium sulphate decahydrate a few mm wide connected together by composite eutectic solid.

dendrites in the mushy layer was denser than the solution, and nucleation sites for crystallization may have been provided by a few small crystals that settled from the mushy layer.

The crystallization at the floor depleted the melt of solute locally to leave a buoyant residual liquid which caused compositional convection of the whole liquid region. The convection of solvent away from the growing crystals allowed for very efficient growth into the supersaturated liquid which we model by assuming that the crystals grew sufficiently rapidly to restore thermodynamic equilibrium to the liquid. This gives an upper bound on the rate of growth of the crystals on the floor, but we shall see that in fact it gives a good approximation for the observed growth. There is still a kinetic undercooling at the interface between mush and liquid as illustrated in the temperature profile of Fig. 2b.

If the crystals on the floor are assumed to occupy a solid layer of thickness $h_f(t)$ then global conservation of solute demands that

$$\dot{h}_f = \frac{H - h_i - h_f}{C_\beta - C_1} \dot{C}_1 \quad (14)$$

where C_1 is the composition, now changing, of the liquid region. Our assumption that the secondary crystal growth restores equilibrium to the convecting liquid can be expressed by

$$T_1 = T_L(C_1) \quad (15)$$

and the latent heat released modifies equation (7), which becomes

$$\mathcal{L}_\beta \dot{h}_f - c_l(H - h_i - h_f) \dot{T}_1 - c_\beta h_f \dot{T}_1 = F_T \quad (16)$$

Although the release of solvent by the secondary crystal growth has an obvious direct effect on the liquid region, it also has an indirect effect on the evolution of the mushy layer. Even though there is no flux of solvent across the interface between mush and liquid, the changing composition of the liquid region means that the composition of the solution incorporated into the advancing mushy layer decreases with time. Consequently, the bulk composition of the mushy layer (including both liquid and solid phases) is a function of height. In fact, as we have neglected vertical transport of solute within the mushy layer, the bulk, horizontally averaged, composition $C_H(z)$ is given simply by the composition that the liquid region had at time t_i when the position of the interface between mush and liquid h_i was equal to z . That is,

$$C_H(z) = C_l(t_i) \quad \text{where } h_i(t_i) = z \quad (17)$$

One effect of the variation in $C_H(z)$ is to alter the solid fraction within the mushy layer

$$\phi = \frac{C_H(z) - C}{C_\beta - C} \quad (18)$$

which affects both the thermal properties given by equations (2) and (3) and the internal release of latent heat as expressed by equation (12). These changes all affect the rates of evolution of the system.

Compositional stratification

A more important consequence of the secondary solidification is that it provides an explanation for the compositional stratification that is often observed when binary melts are completely solidified. To investigate this effect quantitatively we carried out experimental and theoretical investigations of systems in which the upper cooled boundary was maintained at a temperature T_b that was lower than the eutectic temperature T_e . Figure 2b shows a schematic diagram of such a system. A composite solid made up of crystals of the two pure end members of composition C_α and C_β occupies the region $0 \leq z < h_e(t)$. Heat transfer across the layer occurs solely by conduction. At very early times $T_i < T_e$ and there is no mushy layer. For most of the evolution, however, the temperature in this composite

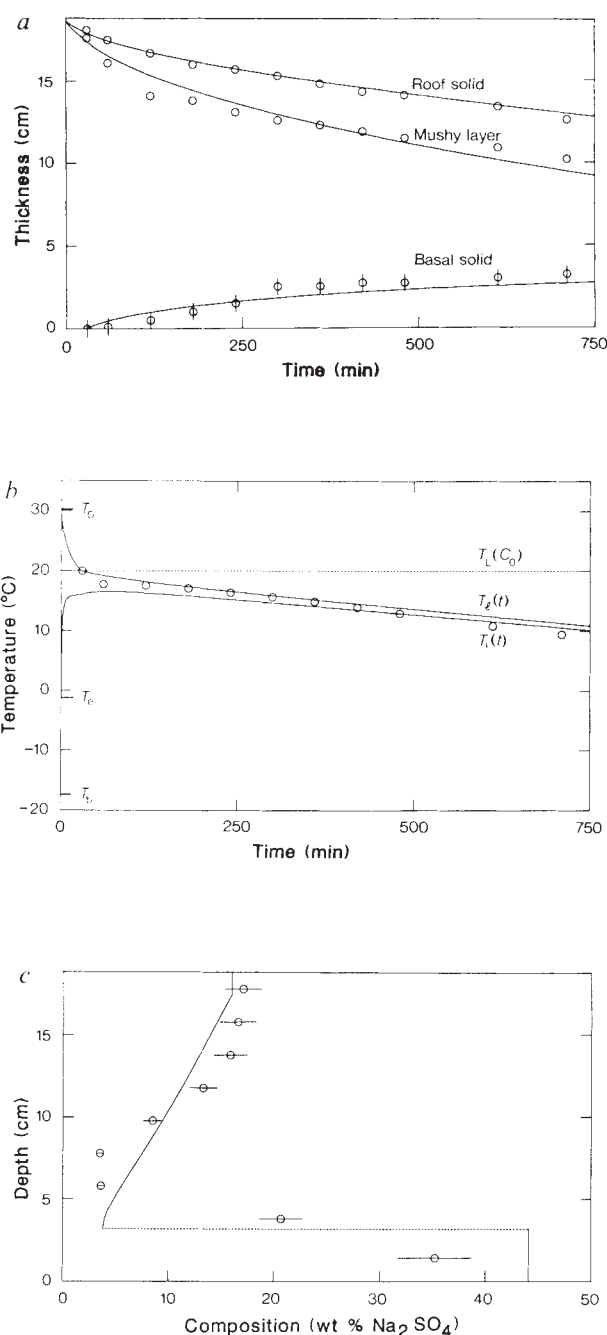


FIG. 5 a, The thickness of the roof solid $h_e(t)$ (upper curve) and mushy layer $h_i(t)$ (central curve) measured from the top of the container (height 18.8 cm) and the basal solid $h_f(t)$ (lower curve) measured from the floor of the tank for the complete solidification of an aqueous solution of sodium sulphate. Circles are experimental data. b, The temperature of the liquid $T_L(t)$ and of the mush-liquid interface $T_i(t)$ as functions of time. The circles show experimental values of the liquid temperature. c, The compositional zonation of the final solid block as predicted by the theory and as measured in the experiment. Some error in the experimental values arises due to the melting of the solid while it is being cut into the 2-cm-thick horizontal sections for sampling. This has been indicated by the error bars. In the calculations $c_l = 4.1 \text{ J cm}^{-3} \text{ } ^\circ\text{C}^{-1}$, $c_\beta = 2.66 \text{ J cm}^{-3} \text{ } ^\circ\text{C}^{-1}$, $\mathcal{L}_\beta = 337 \text{ J cm}^{-3}$, $\mathcal{L}_\alpha = 306 \text{ J cm}^{-3}$, $\nu = 0.023 \text{ cm}^2 \text{ s}^{-1}$, $k_l = 0.0059 \text{ W cm}^{-1} \text{ } ^\circ\text{C}^{-1}$, $k_\beta = 0.008 \text{ W cm}^{-1} \text{ } ^\circ\text{C}^{-1}$, $k_\alpha = 0.022 \text{ W cm}^{-1} \text{ } ^\circ\text{C}^{-1}$, $\lambda = 0.056$, $\alpha = (5.0 - (20 - T_i) \times 0.24) \times 10^{-4} \text{ } ^\circ\text{C}^{-1}$ where T_i in $^\circ\text{C}$ is the liquidus temperature of the melt. The eutectic point is $T_e = -1.2^\circ\text{C}$. $C_e = 3.8 \text{ wt\% Na}_2\text{SO}_4$ and the composition of the solid dendrites, made of $\text{Na}_2\text{SO}_4 \cdot 10 \text{ H}_2\text{O}$, is $C_\beta = 44.1 \text{ wt\% Na}_2\text{SO}_4$. The initial melt temperature and composition were 30.5°C and $16 \text{ wt\% Na}_2\text{SO}_4$. The liquidus curve for concentrations above C_e was calculated with a cubic spline by using data from ref. 24. The kinetic growth parameter used was $\mathcal{G} = 1.5 \times 10^{-4} \text{ cm s}^{-1} \text{ } ^\circ\text{C}^{-1}$.

layer rises from T_b at $z=0$ to T_e at $z=h_e$. Below the eutectic front $z=h_e$, the system looks much as before: a stagnant mushy layer extends from $z=h_e$ to $z=h_i$ and lies above a convecting liquid region. Secondary crystals growing near the base of the container fill a depth h_f from the floor. This structure can be seen in the photographs in Fig. 4a, b. The eutectic front $z=h_e$ eventually reaches the base of the container, by which time the whole system is solidified. Once this had occurred in the experiments the whole solidified block, shown in Fig. 4c, was cut into horizontal sections and the bulk composition of each horizontal section was measured.

Figure 5 shows the results. Figure 5c shows that the mean composition decreases with depth from the top of the sample, which reflects the decreasing composition of the liquid region with time during the experiment. There is a jump in mean composition at the height where the interface between mush and liquid met the crystal layer growing up from the floor. The compositional discontinuity also marks a discontinuity in crystal morphology from vertically oriented dendritic crystals grown from the roof to randomly oriented smaller crystals grown at the floor. This is the so-called columnar-equiaxed transition, previously described by metallurgists⁹. Below this discontinuity the bulk composition is essentially uniform and equal to the composition of the pure component C_p . A smaller value of $\mathcal{G}$ than the one appropriate to the isopropanol system was needed to fit the data. This reflects the fact that crystals of sodium sulphate decahydrate are more faceted than ice crystals and require a greater supersaturation for a given growth rate.

Discussion

At a fundamental level the agreement between the results of our theoretical model and experiments validates our description of the mushy layer and its simulation as a continuum. There are many practical applications of this macroscopic approach to various situations considered by crystal growers and metallurgists, as discussed by Brice¹⁰ and Brown¹¹. There are also important geological applications, for example to the understanding of the cooling and solidification of lava flows. Basaltic lava lakes, cooled by the air¹², komatiitic lavas ponded on the sea floor² and magma chambers subject to hydrothermal cooling at the roof¹³ are all examples in which the strongest cooling is from above and where the viscosity of the lava or magma is sufficiently low for them to convect turbulently. Equilibrium models of solidification will accurately predict the removal of any superheat from the lava and the initial formation of a dendritic crust. In such models, however, the crust buffers the interior of the lava from cooling below its initial liquidus temperature. By contrast, these models show that the coupling of fluid mechanical and disequilibrium effects can cause additional crystallization of the lava, either at the base of the flow or in its interior. This secondary solidification effects a change in the

composition of the lava, which lowers the liquidus temperature and allows cooling and convection to continue.

In fact, turbulent convection can occur in a magma chamber even when there is no initial superheat. In such a case, before the onset of convection, both the depth of the mushy layer h and the thickness of the boundary layer below the mush-liquid interface δ , have magnitudes of order $(\kappa t)^{1/2}$, once the time elapsed since emplacement of the magma t is greater than τ_g . Accordingly, equation (13) shows that there is a kinetic undercooling ΔT of magnitude $\mathcal{G}^{-1}(\kappa/t)^{1/2}$. The local Rayleigh number based on δ and ΔT will exceed the critical value for convective instability Ra_c after a time of order $\tau_c = (\mathcal{G}\nu/\alpha g \kappa) Ra_c$. If $\tau_c \ll \tau_\kappa$ there is sufficient time for breakdown of the boundary layer to generate turbulent convection in the melt. This condition is satisfied once the global Rayleigh number, based on the full depth of the chamber H and ΔT , is much greater than Ra_c , which is expressed by

$$\frac{\alpha g H^2}{\mathcal{G} \nu} \gg Ra_c (\approx 10^3) \quad (19)$$

A similar inequality was derived by Brandeis and Jaupart¹⁴ for disequilibrium leading to nucleation of new crystals in the thermal boundary layer ahead of the solidification front. Alternatively, if there is some superheat initially such that the global Rayleigh number, based on H and the initial superheat, is sufficiently large then turbulent convection will occur even without the effects of disequilibrium².

Our study demonstrates further how the changing composition of the melt results in a stratification of the bulk composition of the mushy layer. It also provides a mechanism for the redistribution of solute during the complete solidification of an alloy cooled from above. Such macrosegregation is observed in completely solidified ingots and in igneous rocks¹⁵.

We hope to extend our concepts to address more complicated and naturally realistic situations. For example, an additional geometrical dimension is introduced when the cooling and crystallization occurs at a side wall¹⁶. Horizontal temperature and compositional gradients drive a predominantly vertical flow adjacent to and through the mushy layer. Controlled experiments^{17,18}, in which cooling and crystallization took place at a sloping boundary, have shown dramatic differences between the forms of motion and crystallization at the upper and lower surfaces. The nature of the convection and its interaction with crystal growth would additionally be influenced by an initial stratification in the melt. Crystals and melt can interact in a fundamentally different way than considered here if the crystals are free to be swept along with any motion in the melt. Such systems have been called 'slurries'¹⁹ in contrast to the mushy regions considered here. Further studies are under way to determine what portion of a slurry is mobile and what form of continuum mechanics, if any, is applicable to such flows. □

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- Huppert, H. E. & Worster, M. G. *Nature* **314**, 703–707 (1985).
- Turner, J. S., Huppert, H. E. & Sparks, R. S. J. *J. Petrology* **27**, 397–437 (1986).
- Flemings, M. C. in *Modeling of Casting and Welding Processes* (eds Brody, H. D. & Arpetian, D.) 533–548 (The Metallurgical Society of the American Institute of Mining, Metallurgical and Petroleum Engineers, Warrendale, 1981).
- Hills, R. N., Loper, D. E. & Roberts, P. H. *Q. J. Mech. appl. Math.* **36**, 505–539 (1983).
- Worster, M. G. *J. Fluid Mech.* **167**, 481–501 (1986).
- Batchelor, G. K. *A. Rev. Fluid Mech.* **6**, 227–255 (1974).
- Turner, J. S. *Buoyancy Effects in Fluids* (Cambridge University Press, 1979).
- Kurz, W. & Fisher, D. J. *Fundamentals of Solidification* (Trans Tech Publications, Aedermannsdorf, 1986).
- Flood, S. C. & Hunt, J. D. *Appl. Sci. Res.* **44**, 27–42 (1987).
- Brice, J. C. *Crystal Growth Processes* (Blackie, Glasgow, 1986).
- Brown, R. A. *A.I.Ch.E. J.* **34**, 881–911 (1988).
- Turcotte, D. L. & Schubert, G. *Geodynamics* (Wiley, New York, 1982).
- Norton, D. & Taylor, H. P. *J. Petrology* **20**, 421–486 (1979).
- Brandeis, G. & Jaupart, C. *Earth planet. Sci. Lett.* **77**, 345–361 (1986).
- Shirley, D. N. *J. Petrology* **28**, 835–866 (1987).
- Nilson, R. H., McBirney, A. R. & Baker, B. H. *J. Volcan. geotherm. Res.* **24**, 25–54 (1985).

- Huppert, H. E., Sparks, R. S. J., Wilson, J. R. & Hallworth, M. A. *Earth planet. Sci. Lett.* **79**, 319–328 (1986).
- Huppert, H. E., Sparks, R. S. J., Wilson, J. R., Hallworth, M. A. & Leitch, A. M. in *Origins of Igneous Layering* (ed. Parsons, I.) 539–568 (Reidel, Dordrecht, 1987).
- Roberts, P. H. & Loper, D. E. in *Structure and Dynamics of Partially Solidified Systems* (ed. Loper, D. E.) 229–290 (Martinus Nijhoff, Dordrecht, 1987).
- Abegg, R. Z. *phys. Chem.* **15**, 209–261 (1894).
- Denton, R. A. & Wood, I. R. *Int. J. Heat Mass Transf.* **22**, 1339–1346 (1979).
- Kaye, G. W. C. & Laby, T. H. *Tables of Physical and Chemical Constants and Some Mathematical Functions* (Longman, London and New York, 1973).
- Vargaftik, N. B. *Tables on the Thermodynamic Properties of Liquids and Gases* (Wiley, New York, 1975).
- Washburn, E. W. (ed.) *International Critical Tables of Numerical Data: Physics, Chemistry and Technology* (McGraw-Hill, New York and London, 1926).
- Weast, R. C. (ed.) *CRC Handbook of Chemistry and Physics* (Chemical Rubber Co., Cleveland, 1971).

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Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the bicoid morphogen

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The maternal gene *bicoid* is a key component of the system that determines the pattern of the anterior half of *Drosophila* embryos. The bicoid protein forms a concentration gradient in early embryos, and is known to bind DNA. Specific binding sites are now shown to confer expression in a region of the embryo that depends on their affinity for bicoid protein: sites of high affinity allow expression further down the bicoid protein gradient than sites of low affinity.

THE maternal gene *bicoid* (*bcd*) determines pattern in the anterior half of the *Drosophila* embryo¹. *Bicoid* messenger RNA is localized at the anterior pole of the egg and early embryo^{2,3}. During the syncytial cleavages, *bcd* protein forms a concentration gradient with a maximum at the anterior tip of the embryo: the concentration of *bcd* protein determines position on the blastoderm fate map^{4,5}.

The *bcd* protein contains a homeodomain and acts as a transcriptional activator of the zygotic gap gene *hunchback* (*hb*); *hb* is expressed in a broad domain in the anterior half of the blastoderm embryo^{8,9}. The *bcd* protein binds to several sites upstream of the *hb* promoter, thereby activating transcription of the gene^{6,7}. Studies using germ-line transformation of *hb lacZ* promoter fusions delimited the regulatory elements necessary for the correct spatial expression of the anterior *hb* domain to a 700-base-pair (bp) fragment, including 300 bp of upstream sequences¹⁰.

We now report the use of P-element-mediated germ-line transformation to analyse further the function of the *bcd* binding sites in determining the spatial limits of target gene expression in the early *Drosophila* embryo. We show that high-affinity binding sites for *bcd* fused to the HSP 70 promoter are sufficient to generate an expression domain over the anterior half of the embryo, similar to that seen for *hb*. In contrast, when low-affinity *bcd* binding sites are fused to the HSP 70 promoter, expression is restricted to the anterior quarter of the early embryo. Thus we demonstrate that the maternally derived gradient of *bcd* can define more than one domain of zygotic gene expression by variation of binding-site affinity.

The *hb* regulatory region

A fragment of 300 bp upstream of the *hb* transcription start site contains three binding sites of relatively high affinity for *bcd* (A1, -282 to -274 CGTAATCCC; A2, -172 to -164 TCTAATCCA; A3, -67 to -59 TCTAATCCC) defining a consensus binding sequence TCTAATCCC. Three more sites are bound with rather low affinity, each deviating by four bases from the consensus sequence (X1, -241 to -233 TGCTAAGCT; X2, -225 to -217 CGCTAAGCT; X3, -212 to -204 GATCATCCA) (ref. 6). We constructed fusion genes that contain

upstream sequences and the transcription start site of the *hb* gene fused to the coding region for β -galactosidase to detect the expression of the fusion gene after germ-line transformation (Fig. 1; Table 1). After successively deleting *bcd* binding sites we observed three effects on the expression of β -galactosidase: first, the level of expression is strongly reduced with each additional site deleted; second, the posterior border of the expression domain appears to be shifted towards the anterior (P-transformation construct pThb1, 51%; pThb2, 60%; and pThb3, 65% egg length; anterior tip is 100%); and third, the posterior border of expression becomes less sharp, having a rather shallow graded form when just one site is present. There is no expression detectable in preblastoderm stages in the absence of any *bcd* binding site (pThb4; see Table 1). The expression patterns observed are dependent on *bcd*, because embryos from females homozygous for *bcd*^{E1} (ref. 1) show no lacZ expression. In addition, embryos from heterozygous *bcd*^{E1} females have expression domains which are shifted towards the anterior (posterior borders of expression: pThb1, 60%; pThb2, 65%; pThb3, 70% ($\pm 4\%$)). This resembles the *bcd* gene-dosage-dependent shift of other positions on the fate map^{1,5}. We conclude that the binding sites for *bcd* contribute in an additive or even synergistic way to the level of *hb* expression. The apparent shift towards the posterior of the embryo and the more sharp posterior border of expression with increasing number of binding sites could be explained by the increased level of expression, a cooperative effect of binding (see below) and/or by small differences in the binding affinities of the A1, A2 and A3 sites for *bcd*.

To test if there is a specific requirement for the proximal part of the *hb* promoter itself, we replaced the *hb* promoter (-55 to +107) with the heterologous HSP 70 promoter (-50 to +90) (ref. 11). The HSP 70 promoter alone is not normally used in the embryo (pThb14; Fig. 3 and Table 1), but is active in the early embryo when fused with *hb* -298 to -55 upstream sequences (pThb9; Fig. 3). But the level of expression appears to be lower and the posterior border is slightly shifted towards the anterior compared with the expression from the natural *hb* promoter. The same is true when a larger *hb* upstream region (-1348 to -55) is fused to the HSP 70 promoter (pThb5; Fig. 2). The HSP 70 promoter might be less active in early embryos than the *hb* promoter, and the pWHL vector we use carries a second HSP 70 promoter (that drives the expression of the *white* gene) immediately 5' to the sequences drawn in the figures, a constellation which might reduce the level of β -galactosidase expression further.

The *hb* (-298 to -55) upstream element acts as an enhancer-like element (Fig. 3 and Table 1) because the same spatial expression pattern is observed when it is fused in reverse orientation to the HSP 70 promoter (pThb6); when fused 1 kilobase (kb) upstream of the HSP 70 promoter the expression is only slightly reduced compared to the normal position. Fusing two copies of this element to the HSP 70 promoter (pThb8) enhances the expression level and also shifts the border of expression towards the posterior to ~50% of the length of the egg.

Bicoid is not the only factor that influences the anterior domain of *hb* expression. The retraction of expression from the anterior tip at late stage 14 appears to be dependent on the terminal-group genes, like *torso*^{9,22}. This retraction is also observed in the *lacZ* patterns of pThb1 to pThb3; it does not occur when the embryos derive from females homozygous for *tor*^{XR1} (ref. 12, only examined with pThb2 insertions). These embryos also have a slight shift towards the anterior of the blastoderm fate map²², detectable by the shifted expression of pair-rule genes as well as the anterior shift (~5%) of the posterior border of our insertion pThb2. Further, in embryos heterozygous for *hb*^{FB} the posterior border of expression from a pThb1 insertion is shifted slightly (~4%) towards the anterior, as is the wild-type *hb* expression. Tautz⁹ discussed a possible autocatalytic circuit. But in homozygous *hb*^{FB} embryos, a strong allele, *lacZ* expression from pThb1 insertions is not changed when compared to heterozygous embryos. In summary, we conclude that the concentration of *bcd* provides the initial spatial cues for *hb* expression, whereas the establishment of a stable expression domain *in vivo* is likely to involve additional factors.

High and low affinity binding sites

To test whether the identified *bcd* binding sites are sufficient to confer a spatially restricted expression to a gene, we constructed fusion genes that have multiple copies of a 25-bp sequence containing the *bcd* binding site A3 upstream of the HSP 70 promoter and the *lacZ* coding region (Fig. 3, Table 1). Fusion genes with three (pThb10) or four (pThb11) A3 binding sites give rise to a spatially restricted expression from 100–65% and 100–58% egg length, respectively. Thus, apparently no other regulatory upstream elements are required for the generation of a *hb*-like expression pattern. The expression is weaker and does not extend as far posteriorly as the expression from the *hb* promoter with the equivalent number of binding sites in the

natural upstream element.

The weak binding sites X1, X2 and X3, when fused to a HSP 70 *lacZ* gene, define an expression domain in the anterior quarter of the embryo (pThb12, 13, 15 and 16, Figs 3, 4 and Table 1). An increase in the number of binding sites (3–9) increases the expression level and sharpens the posterior border of expression but does not significantly shift the posterior border towards the posterior. In order to investigate the nature of the small apparent posterior shifts of expression, we performed densitometric measurements of the anti- β -galactosidase immunostain intensities in embryos with fusion genes carrying 3, 6 or 9 low affinity binding sites for *bcd* protein (Fig. 4). In contrast to the visual impressions for the stainings, these measurements show, that the apparent posterior shift is mainly due to the increased level of expression and the sharpened border rather than to a shift of the expression domain itself. Similar results were obtained for the high affinity binding sites (not shown). There is no indication of a special function of the low affinity binding sites in the natural *hb* promoter, as the *hb* expression is not higher in the anterior quarter of the embryo⁹. These binding sites might contribute to an overall high level of *hb* expression by allowing cooperative interactions between binding sites.

The expression patterns that are caused by the presence of either high-1 or low-affinity *bcd* binding sites (pThb11 and pThb13, respectively) in the upstream region of the HSP 70 promoter are both dependent on *bcd*, because embryos from *bcd*^{E1} homozygous females show no expression. Furthermore the expression patterns are influenced by the maternal *bcd* gene dosage: they extend from 100–68% (pThb11) and 100–85% (pThb13), respectively, in embryos from females heterozygous for *bcd*^{E1}. The expression patterns of both the above-mentioned fusion genes are not altered in a *tor* mutant maternal genetic background, excluding an influence of the terminal-group genes.

TABLE 1 Expression of fusion genes in the *Drosophila* embryo

Name	<i>hb</i> sequences	Number of insertions	Domain of expression (% egg length)	Relative intensity of expression
<i>hb</i> promoter				
pThb1	(–298 to +107)	8	51–100 (±2)	Very strong
pThb2	(–234 to +107)	17	60–100 (±4)	Intermediate
pThb3	(–94 to +107)	7	65–100 (±5)*	Very weak
pThb4	(–55 to +107)	11	None*	
HSP 70 promoter				
pThb5	(–1348 to –55)	4	55–100 (±3)	Strong
pThb6	(–55 to –298)	6 (1)	55–100 (±3)	Strong
pThb7	(–55 to –1348)	7	58–100 (±4)	Intermediate
pThb8	(2 × –55 to –298)	4	51–100 (±3)	Very strong
pThb9	(–298 to –55)	6	55–100 (±3)	Strong
pThb10	(3 times A3)	6 (1)	65–100 (±3)	Weak
pThb11	(4 times A3)	5 (1)	58–100 (±4)	Intermediate
pThb12	(X1 X2 X3)	4	79–100 (±3)	Weak
pThb13	(X1 X1 X2 X3)	5	77–100 (±3)	Intermediate
pThb15	(2 times X1-X3)	3	72–100 (±3)	Strong
pThb16	(3 times X1-X3)	7	70–100 (±3)	Strong
pThb14	(None)	3	None	

The table quantifies the data described in Figs 1 to 4. Several pThb1 lines had an additional expression in the mesoderm following gastrulation, similar to the one described and discussed by Schröder *et al.* (ref. 10) for fusion genes with longer upstream regions. The relative intensities of expression are based on visual estimates of the intensities of immunostaining using identical conditions for the immunostain reaction. Different insertions of one fusion-gene construct varied only slightly in the intensity of expression. When determining the posterior borders of expression, we cannot exclude that a very high level of expression might have resulted in a measurement of borders marginally more posterior than where the actual transcription took place, because the β -galactosidase diffuses during syncytial blastoderm stages (see also Fig. 4). Number of insertions gives the number of independent insertions analysed for their pattern of fusion-gene expression. The number of insertions that did not show an expression is given in brackets. The extent of the expression domain was measured from camera lucida drawings of immunostained embryos (three for each insertion line). The anterior tip of the egg is defined as 100% egg length; the mean error of the determinations is given in brackets. *Plasmids pThb3 and pThb4: at gastrulation, a weak stripe of expression appeared in the head region, ranging from ~75 to 85% egg length. This is most probably an expression artefact due to sequence elements in the pCaSpeR-AUG- β gal vector, as similar patterns have been observed with other inserts (D. Weigel, personal communication). Due to the presence of high levels of β -galactosidase in the same region, this expression cannot be identified in pThb1 and pThb2 fusion-gene insertions.

FIG. 1 Deletion analysis of the upstream region of the zygotic 2.9-kb *hb* transcript. Fragments of the *hb* upstream region with a sequentially reduced number of bcd binding sites were fused to the lacZ coding region at position +107 with respect to the transcription start site of the 2.9-kb *hb* transcript⁸. The fusion genes were introduced into the germ line of *Drosophila* by P-element-mediated transformation¹⁷. Left panel: schematic maps of the fusion-gene constructs. Numbers above the line give positions with respect to the start of transcription. A1, A2 and A3 refer to the high-affinity binding sites, X1, X2 and X3 to the low-affinity binding sites for bcd (see text and ref. 6) pThb1 to pThb4: names of the fusion genes. Right panel: Expression pattern of the *hb* LacZ fusion genes in stage-14 embryos¹⁸ as revealed by the immunostaining of whole embryos with anti- β -galactosidase. Pictures show optical sagittal sections using Normarski optics (for quantification see Table 1).

METHODS. Generation of the fusion genes: The *hb* fragments⁸ were isolated from hbCAT-298 (for pThb1), hbCAT-231 (pThb2), hbCAT-94 (pThb3) and hbCAT-55 (pThb4) (ref. 6) by digestion with *Hind* III, Klenow polymerase treatment and *Bam*HI digestion. The P-transformation vector pCaSpeR-AUG- β gal (ref. 19) was cut at the *Eco*RI site, Klenow polymerase treated and cut with *Bam*HI. P-element-mediated transformation: fusion-gene plasmids were dissolved in water at 0.4 mg ml⁻¹ together with 0.1 mg ml⁻¹ helper-plasmid Delta 2-3 (ref. 20). Injection into homozygous *white*¹ embryos and further processing was according to standard procedures²¹. Rabbit anti- β -galactosidase antibodies (gift of Dr U. Gaul) and the vectastain elite ABC detection system (vector laboratories) were used to detect β -galactosidase.

FIG. 2 A fragment of the *hb* upstream region containing bcd binding sites behaves as an enhancer-like element. Fragments of the *hb* gene were fused in different orientations 5' to the HSP 70 promoter located in front of the β -galactosidase coding region. The fusion genes were introduced into the germ line by P-element-mediated transformation. Left panel: schematic maps of the fusion genes. The orientation of the A-type binding sites is indicated by triangles; X-type binding sites are palindromic. Right panel: expression pattern as visualized by immunostaining with anti- β -galactosidase. For details see Fig. 1.

METHODS. Generation of fusion genes: fragments of the *hb* gene⁸ were prepared by *Mlu*I and *Hind*III digest of hbCAT-298 (ref. 6; for pThb6, pThb8 and pThb9, Fig. 3) or *Mlu*I and *Nde*I digest of hbCAT-2, 172 (ref. 6; for pThb5 and pThb7) and subsequent treatment with Klenow Fragment of DNA polymerase and cloned into the pWHL vector which had been linearized with *Stu*I. Plasmid pWHL (obtained from Dr F. Michiels, Martinsried, West Germany) is a pW8-based P-transformation vector: on one side of the polylinker (containing the *Stu*I site) a HSP 70 promoter initiates the expression of the *white* coding region, and on the other a second HSP 70 promoter (-50 to +90) the expression of the lacZ coding region. The orientation and number of inserts was verified by restriction digests. Transformation and immunostaining were performed as in Fig. 1.

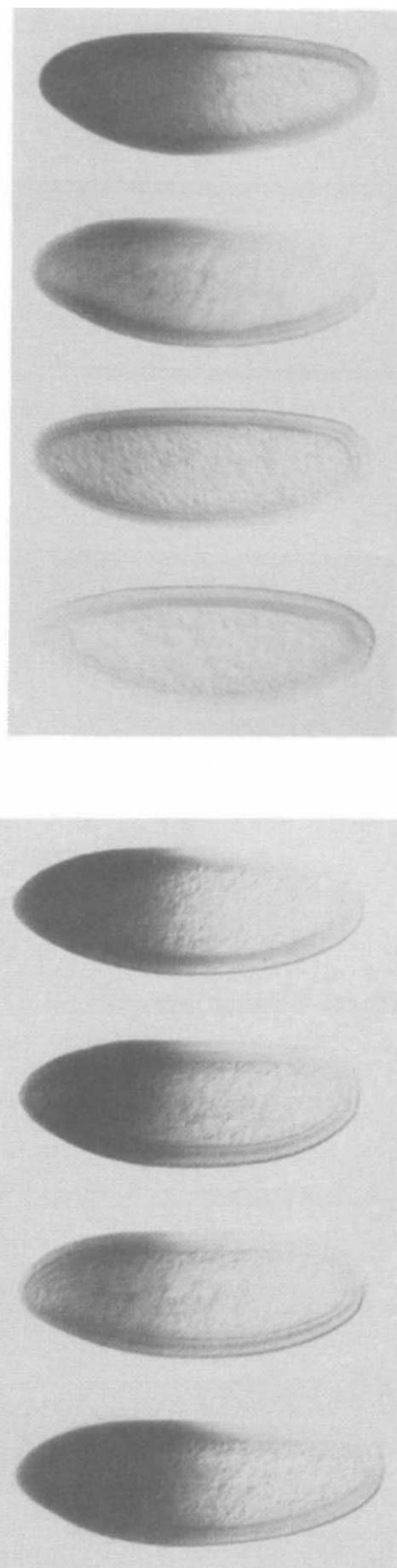
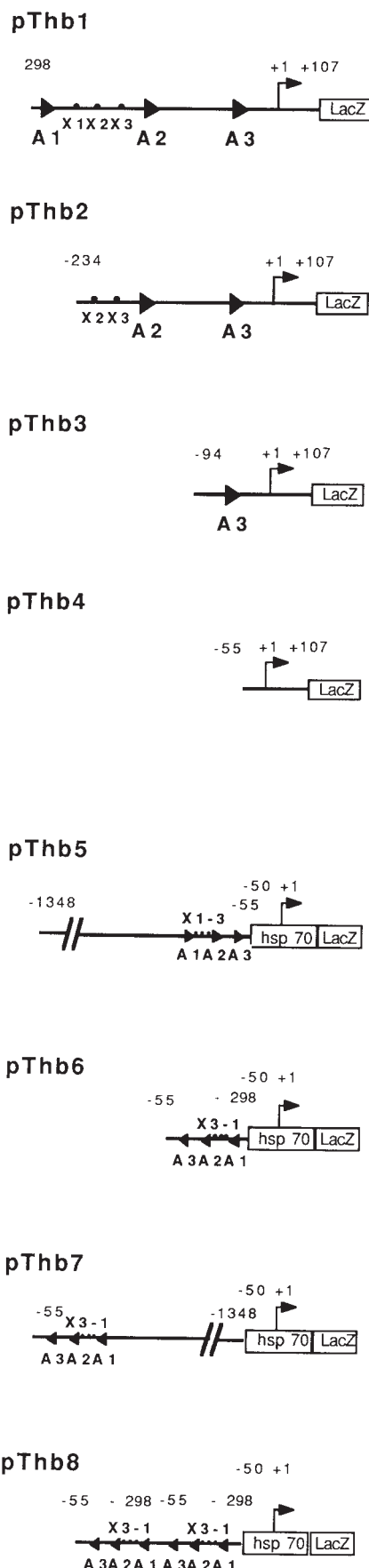
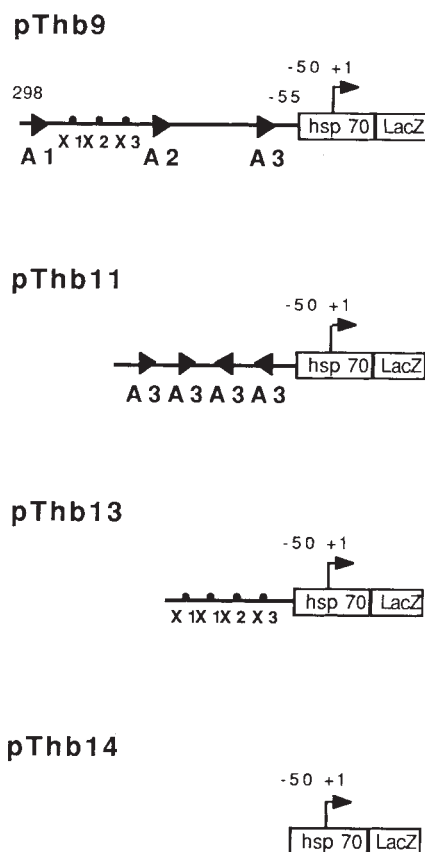


FIG. 3 Bicoid binding sites are sufficient to define distinct domains of fusion-gene expression in the early *Drosophila* embryo. The *hb* -298 to -55 upstream region or short DNA fragments containing bcd binding sites were fused 5' to the HSP 70 promoter located in 5' to the β -galactosidase coding region. Fusion genes were introduced into the germ line by P-element-mediated transformation. Left panel: schematic maps of the fusion-gene constructs. pThb 11: the number and orientation of the A3 bcd binding sites are indicated by triangles; pThb 13: X-type binding sites are palindromic. pThb14: control, vector without insert. Right panel: expression pattern as visualized by immunostaining with anti- β -galactosidase. For details see Fig. 1. METHODS. Generation of fusion genes. The construction of pThb9 is described in the legend to Fig. 2. Plasmids pThb10 (Table 1) and pThb11: a phosphorylated 25-bp oligonucleotide-derived duplex DNA with the A3 binding site (-74 to -50: CTGCCCATCTAATCCCTTGACGCTG; binding consensus printed bold) was inserted into pUC 18, cut with *Sma*I, and the number and orientation of the inserts were determined by DNA sequencing (Sequenase, USB). Plasmid pUC18pThb10 is identical to pUC18pThb11 except that it lacks the 5'-most binding site. The *Eco*RI (filled in) *Sph*I fragment from these pUC derivatives was cloned into pWHL cut with *Stu*I and *Sph*I. pThb12 (Table 1) and pThb13: an oligonucleotide-derived phosphorylated duplex DNA with *hb* sequences from -244 to -205 (containing the X1, X2 and X3 binding sites), an *Eco*RI site at the 5' and a *Bam*HI site at the 3' end was inserted into pUC 18 cut with *Eco*RI and *Bam*HI (pThb12) or in two copies into the *Bam*HI site of pUC 18 (pThb13). DNA sequence analysis revealed that pUC18pThb13 had a mutation such that only the X1, X1-X2-X3 sites remained intact. The fragments were then subcloned into



the pWHL vector. Transformation and immunostaining were performed as in Fig. 1.

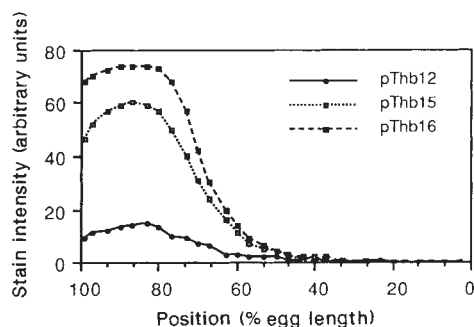


FIG. 4 Distribution of β -galactosidase expressed from fusion genes carrying three (pThb12), six (pThb15) or nine (pThb16) low affinity bcd binding sites in the upstream region. Drawing the three curves each to a scale that the maximal values are identical demonstrates, that, apart from different absolute values, the profiles of the curves are very similar. METHODS. Fusion genes carrying one (pThb12), two (pThb15) or three (pThb16) copies of the X1-X2-X3 *hb* sequence element in tandem array upstream of the HSP 70 promoter and the *lacZ* coding region were introduced into the germ line of *Drosophila* by P-element-mediated transformation (see legends to Figs 1 and 3). Embryos were immunostained for β -galactosidase (see Fig. 1). Video images of whole mount embryos (cellular blastoderm stage) were obtained and the stain intensities were recorded along an anterior-posterior midline as described⁴. The graph shows mean values calculated for five embryos of each genotype. Immunostain reactions were not performed in the same but in parallel batches, therefore the distribution profiles rather than the absolute values obtained from measurements of the different genotypes should be compared.

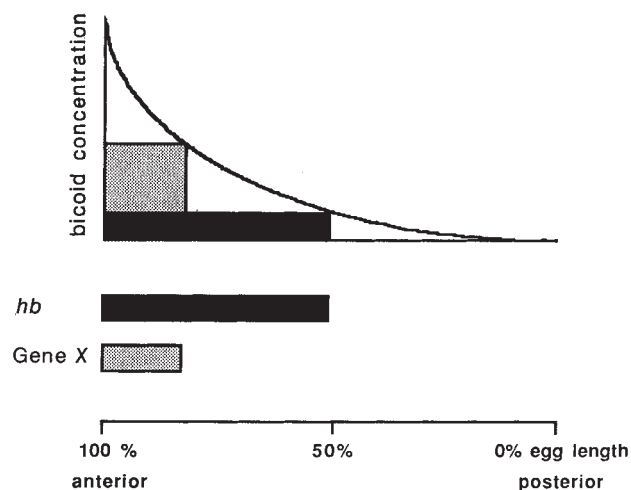


FIG. 5 Model for the specification of distinct domains of zygotic gene expression by the morphogen bcd. Within the graded field of bcd concentration, target genes with low-affinity binding sites (gene X) are expressed at high bcd concentrations only, whereas the ones with high-affinity binding sites (like *hunchback*) are also expressed further posterior at low bcd concentrations. The border of expression for gene X is arbitrarily drawn in the figure and need not correlate with the one of authentic target genes during *Drosophila* embryonic development.

Conclusions

Our data suggest that the function of multiple binding sites in the upstream region of a target gene promoter is not only to assure a sufficiently high level of expression but also to generate sharp borders of expression. The observation that the domain of gene expression apparently ranges slightly more posteriorly when increasing numbers of binding sites are present in the upstream region might predominantly result from the increased expression level and the sharpened border (Fig. 4). The precision of our measurements does not allow to decide whether cooperative binding effects contribute to the sharpening of the border of expression. Cooperative effects result in sigmoidal binding characteristics, allowing a switch between the on- and the off-state within a narrow range of concentrations. Thus, when at a concentration above a certain threshold, bcd could bind efficiently to multiple binding sites, whereas at a lower concentration at positions slightly more towards the posterior it could not bind at any of the sites. The effects of binding sites with higher and lower affinity for bcd on the expression of fusion genes gives us important clues on how the maternal morphogen bcd might define distinct domains of expression of other zygotic genes.

Target genes with high-affinity binding sites, like the *hb* gene, are efficiently expressed even at low bcd concentrations. In contrast, the expression of target genes with low-affinity binding sites, as shown with our fusion gene pThb13, is restricted to more anterior positions of the embryo where higher bcd con-

centrations prevail (Fig. 5). Such a mechanism would translate the smoothly graded information of the maternal bcd gradient into discrete domains of zygotic gene expression (see also refs. 13–15).

Genetic analysis predicts the existence of at least one other target gene (here called gene *X*) besides *hb* for the following reasons. First the defects caused by mutations in the *hb* gene are only part of the *bcd* mutant phenotype^{1,16}. Second, both cytoplasm transplantation experiments¹ and genetic analysis⁵ show that low levels of bcd are sufficient to induce thoracic structures (*hb* level), whereas high levels are necessary for the formation of the head (level of expression of gene *X*). Third, the other maternal group of genes that affect head development, the *torso* group, only deletes the anteriormost part of the head, the acron, while the major part of the head including the regions specified by *hb* and the postulated gene *X*, merely depend on *bcd*. Embryos that lack the maternal signals from the other two systems that affect anterior-posterior development (*oskar-torso*, for the posterior and terminal system, ref. 23) still have a polarity, which has to be specified in at least two steps by the bcd gradient. Thus there is compelling evidence for the existence of at least one other target gene. Our data suggest, on a molecular basis, how bcd could act as a morphogen during early embryogenesis in specifying the domains of *hb* and gene *X* expression. □

Note added in proof. Recently, Struhl *et al.*²⁴ published a similar analysis of the *hb* promoter.

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1. Frohnhofer, H. G. & Nüsslein-Volhard, C. *Nature* **324**, 120–125 (1986).
2. Frigerio, G. *et al.* *Cell* **47**, 735–746 (1986).
3. Berlieth, T. *et al.* *EMBO J.* **7**, 1749–1756 (1988).
4. Driever, W. & Nüsslein-Volhard, C. *Cell* **54**, 83–93 (1988).
5. Driever, W. & Nüsslein-Volhard, C. *Cell* **54**, 95–105 (1988).
6. Driever, W. & Nüsslein-Volhard, C. *Nature* **337**, 138–143 (1989).
7. Ma, J., Driever, W., Ptashne, M. & Nüsslein-Volhard, C. *Nature* (in the press).
8. Tautz, D. *et al.* *Nature* **327**, 383–389 (1987).
9. Tautz, D. *Nature* **332**, 281–284 (1988).
10. Schröder, C., Tautz, D., Seifert, E. & Jäckle, H. *EMBO J.* **7**, 2881–2888 (1988).
11. Karch, F., Török, I. & Tissières, A. *J. molec. Biol.* **148**, 219–230 (1981).
12. Klingler, M., Erdélyi, M., Szabad, J. & Nüsslein-Volhard, C. *Nature* **335**, 275–277 (1988).
13. Lewis, J. H., Slack, J. M. W. & Wolpert, L. *J. theor. Biol.* **65**, 579–590 (1977).
14. Crick, F. *Nature* **225**, 420–422 (1970).
15. Wolpert, L. *J. theor. Biol.* **25**, 1–47 (1969).

16. Lehmann, R. & Nüsslein-Volhard, C. *Dev. Biol.* **119**, 402–417 (1987).
17. Spradling, A. C. & Rubin, G. M. *Science* **218**, 341–347 (1982).
18. Campos-Ortega, J. A. & Hartenstein, V. *The Embryonic Development of Drosophila melanogaster* (Springer, Berlin, 1985).
19. Thummel, C. S., Boulet, A. M. & Lipshitz, H. D. *Gene* **74**, 445–456 (1988).
20. Laski, F. A., Rio, D. C. & Rubin, G. M. *Cell* **44**, 7–19 (1986).
21. Roberts, D. B. (ed.) *Drosophila: A Practical Approach* (IRL Press, Oxford 1986).
22. Schüpbach, T. & Wieschaus, E. *Roux's Arch. dev. Biol.* **195**, 302–317 (1986).
23. Nüsslein-Volhard, C., Frohnhofer, H. G. & Lehmann, R. *Science* **238**, 1675–1681 (1987).
24. Struhl, G. *et al.* *Cell* **57**, 1259–1213 (1989).

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LETTERS TO NATURE

PSR 1820–11: a binary pulsar in a wide and highly eccentric orbit

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HERE we present timing measurements of the pulsar, PSR 1820–11, which show it to be in a binary system with a wide and highly eccentric orbit. This combination of orbital characteristics has not been seen before and presents difficulties for conventional evolutionary scenarios^{1–4}. Pending future measurements of the periastron advance, we cannot place any firm constraints on the nature of the pulsar's companion but we expect it to be another neutron star. Such a binary pulsar might provide further observational evidence for asymmetric supernova explosions in wide binary systems⁵.

The forty pulsars discovered in the Jodrell Bank 1,400 MHz survey (refs 6 and 7; T. R. Clifton, A.G.L., A. W. Jones and J.M., manuscript in preparation) have been the subject of an extensive timing program since August 1985. The results have

provided period derivatives and accurate positions for these pulsars (ref. 8; T. R. Clifton, A.G.L., A. W. Jones and J.M., manuscript in preparation). It also became clear during the analysis that one of them, PSR 1820–11, was orbiting a companion object.

The timing observations on this new binary pulsar were carried out over nearly three years with the 76-m Lovell telescope at Jodrell Bank, using frequencies ranging from 608 to 1,666 MHz. The site arrival times were obtained using standard procedures⁹ and were corrected to the Solar System barycentre using the JPL DE 200 ephemeris^{10,11}. The variation of the pulsar's barycentric period, shown in Fig. 1, is due to the Doppler effects of its binary motion. The orbital period is just under a year and the 'saw-tooth' form of the curve clearly demonstrates the large eccentricity of the orbit. A least-squares fit to pulse phase yielded a sensitive measurement of the pulsar's parameters and these are displayed in Table 1. The timing position agrees with the astrometric position determined at the Very Large Array (VLA) (E. B. Fomalont, W. M. Goss, A.G.L. and R. N. Manchester, personal communication; D. A. Frail, J.M. and A.G.L. personal communication).

These timing results show that PSR 1820–11 has a characteristic age ($P/2\dot{P}$) of 3.2×10^6 yr, making it the youngest known binary pulsar. The surface magnetic field of the pulsar is estimated to be 6.3×10^{11} G (ref. 9) and its dispersion measure

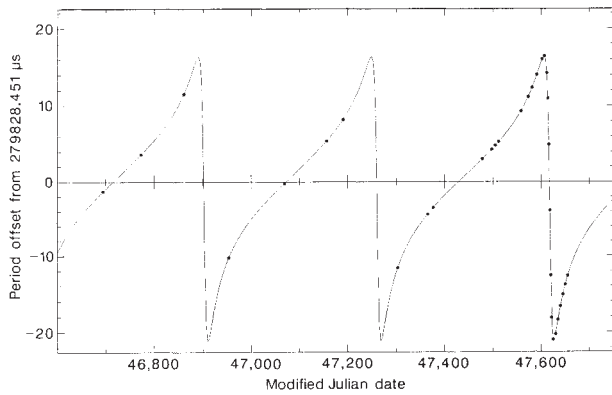


FIG. 1 Plot of pulse period as a function of epoch for PSR 1820-11. The curve, showing the period variation expected from the orbital parameters in Table 1, indicates both high eccentricity and $\omega \approx 90^\circ$. The errors associated with the data are typically one-hundredth of the symbols' diameter.

indicates a distance of ~ 11 kpc (ref. 12).

The nature of a binary pulsar's companion is crucial in understanding the evolution of the system. In the case of PSR 1820-11, however, we can presently only make a few general comments on this object. The mass function of the pulsar, which gives some information on the masses, m_p and m_c , of the pulsar and its companion, is obtained from only two observables, the projected semi-major axis, $a \sin i$, and the orbital period, P_b . It is given by

$$f(m_p, m_c) = \frac{4\pi^2 (a \sin i)^3}{G P_b^2} = \frac{(m_c \sin i)^3}{(m_p + m_c)^2}$$

where i is the inclination of the orbit. For PSR 1820-11, $f(m_p, m_c)$ has the value $0.068 M_\odot$, showing that for a pulsar mass $m_p \approx 1.4 M_\odot$, there is an $\sim 80\%$ chance that the companion mass is $\leq 1.4 M_\odot$ (Fig. 2). Geometrical considerations and the absence of an observed eclipse place no imposing constraints on the size of the companion¹³ because the orbit is so large. The identification of an optical companion seems unlikely. As the pulsar is 11 kpc distant and in the galactic plane, we would expect at least 10 mag of optical extinction. Hence the apparent magnitude of a visual companion would be at least 25 mag fainter than its absolute magnitude. This suggests that even a very luminous main-sequence counterpart would be difficult to detect.

Binary pulsars seem to fall into two groups¹⁻³. The majority of those known are in nearly circular orbits ($e < 0.01$) and have comparatively small mass functions. Their companions are

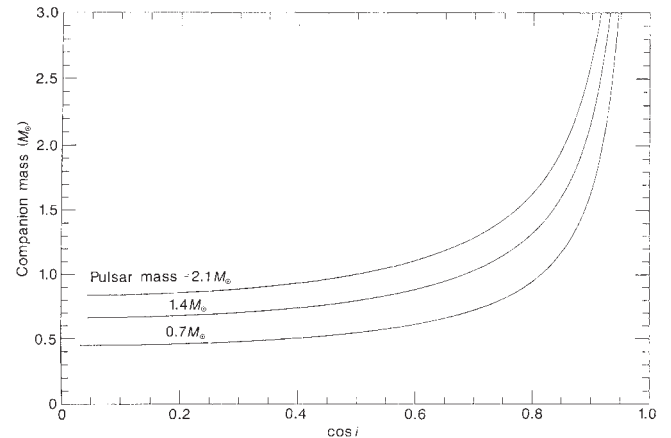


FIG. 2 The mass of the companion, m_c , plotted as a function of $\cos i$ for various neutron-star masses for the observed mass function $f(m_p, m_c) = 0.068 M_\odot$. For $\cos i \leq 0.8$, we see that $m_c \leq 1.4 M_\odot$ if $m_p = 1.4 M_\odot$.

thought to be white dwarfs and such objects have been identified with three binary pulsars^{14,15}. The second category comprises the pulsars PSR 1913+16 and PSR 2303+46 (refs 16, 17), which have large mass functions and large eccentricities ($e > 0.6$), suggesting twin neutron-star systems. Given this dichotomy, we believe the companion of PSR 1820-11 to be another neutron star. Nevertheless, PSR 1820-11 does not fit comfortably into the latter class because its orbital period is anomalously large.

All evolutionary paths leading to a neutron star/neutron star binary involve a 'common-envelope' phase in which a neutron star and the evolved core of a massive star are embedded within a mutual envelope of gas. The high frictional drag associated with this envelope is thought to reduce the orbital period by a few orders of magnitude to values between 1 h and 1 day^{1-4,18}. After this spiral-in, the evolved core may collapse to form another neutron star, the accompanying supernova explosion inducing considerable orbital eccentricity or even completely disrupting the system. This is the scenario invoked to explain the second class of binary pulsars of which PSR 1820-11 seems to be a member. Although PSR 1820-11 is probably not wide enough to have avoided a common-envelope phase, the present periastron separation of the two stars suggests that the pre-supernova orbital period was at least 25 days, which is considerably larger than accepted theory allows.

PSR 1820-11 does, however, provide some support for the recent conclusion of Bailes⁵ that supernova explosions are asymmetric and that pre-supernova orbital periods are typically a few hundred days. With these assumptions, it is possible to predict correctly the incidence of binary pulsars and to reproduce the magnetic moment/transverse-velocity correlation seen in single pulsars¹⁹. These simulations also indicate that massive long-period binary systems might very occasionally survive two supernovae to leave a system similar to PSR 1820-11 (ref. 5). Conventional theory¹⁻⁴ seems to completely exclude the possibility of such binary pulsars.

It is quite possible that the pulsar's companion is something other than a neutron star. A low-mass object such as a hydrogen main-sequence star, a helium star or a white dwarf would, however, prove troublesome when attempting to account for the observed orbit. High-mass companions, such as a massive main-sequence star or a black hole, are unlikely given the low mass function but might be easier to incorporate into standard scenarios²⁰.

One way of distinguishing between the various possible companions would be a measurement of $\dot{\omega}$, the rate of periastron advance^{13,21}. A large main-sequence companion ($r_c \geq 15 R_\odot$) could give a substantial classical contribution to this but it is more likely that the general relativistic contribution, $\dot{\omega}_{gr}$, will dominate as seems to be the case with other binary pulsars of

TABLE 1 Parameters of the PSR 1820-11 system

Pulsar period, P	$279.828.45082 \pm 0.00002 \mu\text{s}$
Epoch	47,400 modified Julian date (MJD)
Pulsar period derivative, $\dot{P}$	$(1.378 \pm 0.001) \times 10^{-15} \text{ s s}^{-1}$
Dispersion measure	$428.4 \pm 0.3 \text{ cm}^{-3} \text{ pc}$
Right ascension (1950.0)	$18 \text{ h } 20 \text{ min } 53.4 \pm 0.1 \text{ s}$
Declination (1950.0)	$-11^\circ 16' 45 \pm 3''$
Orbital period, P_b	$357.7622 \pm 0.0003 \text{ days}$
Projected semi-major axis, $a \sin i$	$200.672 \pm 0.003 \text{ light s}$
Eccentricity, e	0.79462 ± 0.00001
Longitude of periastron, ω	$99.172 \pm 0.002^\circ$
Epoch of periastron, T_0	$47,260.543 \pm 0.001 \text{ MJD}$
Periastron advance, $ \dot{\omega} $	$< 0.01^\circ \text{ yr}^{-1}$
Derived parameters	
Mass function, $f(m_p, m_c)$	$0.068 M_\odot$
Pulsar surface magnetic field, B	$6.3 \times 10^{11} \text{ G}$
Pulsar characteristic age, τ_c	$3.2 \times 10^6 \text{ yr}$
Pulsar distance	11 kpc

high eccentricity^{16,17}. If this new system contains two neutron stars, for instance, $\dot{\omega}_{\text{gr}} \approx 10^{-6} - 10^{-5} \text{ }^\circ \text{yr}^{-1}$, a small value but measurable within ~ 5 years. $\square$

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1. van den Heuvel, E. P. J. in *Accretion-Driven Stellar X-ray Sources* (eds Lewin, W. H. G. & van den Heuvel, E. P. J.) Ch. 8 (Cambridge University Press, 1983).
2. van den Heuvel, E. P. J. & Taam, R. E. *Nature* **309**, 235–237 (1984).
3. van den Heuvel, E. P. J. in *Timing Neutron Stars* (eds Ögelman, H. & van den Heuvel, E. P. J.) 523–548 (Kluwer, Dordrecht, 1989).
4. Dewey, R. J. & Cordes, J. M. *Astrophys. J.* **321**, 780–798 (1987).
5. Bailes, M. *Astrophys. J.* (in the press).
6. Clifton, T. R. thesis, Univ. of Manchester (1985).
7. Clifton, T. R. & Lyne, A. G. *Nature* **320**, 43–45 (1986).
8. McKenna, J. in *Timing Neutron Stars* (eds Ögelman, H. & van den Heuvel, E. P. J.) 143–152 (Kluwer, Dordrecht, 1989).
9. Manchester, R. N. & Taylor, J. H. in *Pulsars* (Freeman, San Francisco, 1977).
10. Standish, E. M. *Astr. Astrophys.* **114**, 297–302 (1982).
11. Backer, D. C. in *Timing Neutron Stars* (eds Ögelman, H. & van den Heuvel, E. P. J.) 3–16 (Kluwer, Dordrecht, 1989).
12. Lyne, A. G., Manchester, R. N. & Taylor, J. H. *Mon. Not. R. astr. Soc.* **213**, 613–639 (1985).
13. Masters, A. R. & Roberts, D. *Astrophys. J.* **195**, L107–L111 (1975).
14. Kulkarni, S. R. *Astrophys. J.* **306**, L85–L89 (1986).
15. Wright, G. A. & Loh, E. D. *Nature* **324**, 127–128 (1986).
16. Taylor, J. H. & Dewey, R. J. *Astrophys. J.* **332**, 770–776 (1988).
17. Taylor, J. H. & Weisberg, J. M. *Astrophys. J.* (in the press).
18. Paczynski, B. in *I.A.U. Symp. No. 73: Close Binaries* (eds Eggleton, P., Mitton, S. & Whelan, J.) 75–80 (Reidel, Dordrecht, 1975).
19. Anderson, B. A. & Lyne, A. G. *Nature* **303**, 597–599 (1983).
20. Habetz, G. M. H. J. *Astr. Astrophys.* **184**, 209–214 (1987).
21. Smarr, L. L. & Blandford, R. D. *Astrophys. J.* **207**, 574–588 (1976).

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First-principles prediction of high-temperature superconductivity in metallic hydrogen

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AT ambient pressure and low temperatures, hydrogen crystallizes in an insulating molecular phase. The possibility of a transition to a metallic structure at high pressures has been the subject of research for over fifty years^{1–6}. Moreover, it has been recognized for some time that metallic hydrogen could be a superconductor², but estimates of its transition temperature vary widely^{4,7,8}. Here we present the first *ab initio* calculation of the electron–phonon coupling constant λ in a distorted hexagonal high-pressure (~ 400 GPa) phase of hydrogen; this first-principles approach has successfully predicted superconductivity in compressed silicon^{9,10}. From the calculated value of λ for this structure, and using standard BCS–Eliashberg^{11–13} theory, the superconducting transition temperature T_c is estimated to be 230 ± 85 K. Thus if metallic hydrogen were to be formed in the laboratory in the structure proposed here or in similar structures, it should be superconducting with the highest T_c yet known.

We predicted recently⁶ that a primitive hexagonal structure of hydrogen may be stable at pressures $p \geq 380$ GPa, and that an insulator–metal transition through band overlap in the molecular phase may occur between 200 and 300 GPa. Although no evidence of a structural transition has yet been found at pressures up to 250 GPa, recent experiments¹⁴ may confirm our results on the existence of the metallic molecular phase. Our calculations suggest that superconductivity could occur in the metallic molecular phase of hydrogen as well as in other metallic phases but we focus here on a specific hexagonal structure that we believe can be stable or metastable. We have not yet calculated the electron–phonon interaction for the metallic molecular

phase because of the complexity of its larger unit cell, but calculations of the phonon frequencies occurring in this structure indicate an average phonon frequency of the same order of magnitude as that of the hexagonal phase. Thus, T_c could be high even in the metallic molecular phase.

Here we report calculations of the electronic band structure, the phonon spectrum and the electron–phonon interaction parameter λ for hydrogen in a distorted primitive hexagonal phase similar to one that we investigated earlier⁶. Our superconductivity studies were motivated by the observation that, within the metallic hexagonal phase, charge density plots show that the bonding remains primarily covalent, that is, anisotropic and directional. It is thought that such bonding is favourable for superconductivity due to enhancement of the electron–phonon interaction by local-field effects¹⁵. We use the frozen-phonon method within a total-energy supercell calculation¹⁶. This method has successfully predicted T_c and its pressure dependence in the highly compressed metallic phases of Si^{9,10}. For hydrogen, we find that the primitive hexagonal phase is unstable (imaginary harmonic phonon frequencies appear) relative to a distorted structure with a tripling of the unit cell along the hexagonal c axis. The distorted structure is stable at a pressure of ~ 400 GPa. We present a description of the properties of this candidate distorted phase including a calculation of λ . This value of λ , together with an estimate of μ^* , the Coulomb interaction parameter, is used in the McMillan equation¹⁷ to estimate T_c . We find that T_c is in the range of 145–300 K with a probable value of 230 K. By calculating the contributions of individual phonon modes to λ , we find that the transition temperature is enhanced by relatively soft phonons with large electron–phonon matrix elements. The caveat accompanying this method is that, because not all possible structures can be tested for stability, the true metallic phase of hydrogen may not be the distorted hexagonal phase. If this is the case, the superconducting transition temperature T_c could differ greatly from these results.

The ground-state properties of hydrogen are calculated using an *ab initio* total-energy approach¹⁸ with a plane-wave basis. In this calculation we have used the true $(1/r)$ potential for hydrogen rather than a pseudopotential. The structural and electronic properties are calculated within the local-density approximation, and the frozen-phonon method is used to calculate the phonon frequencies and electron–phonon matrix elements within supercells¹⁶. The only inputs to these procedures are the atomic number and atomic mass of the hydrogen atom.

The superconducting transition temperature is calculated from the McMillan^{17,19} equation for $\lambda \leq 1.25$:

$$T_c = \frac{\langle \omega \rangle}{1.2} \exp \left(\frac{-1.04(1 + \lambda)}{\lambda - \mu^* - 0.62\lambda\mu^*} \right) \quad (1)$$

For $\lambda > 1.25$, the McMillan equation provides only a lower bound on T_c . In this range of λ , T_c is calculated numerically from Eliashberg strong-coupling theory^{13,19}. The electron–phonon interaction parameter, λ , is an average over the Brillouin zone of the wavevector-dependent coupling parameter $\lambda_{\mathbf{q}\nu}$, summed over the phonon modes ν . The parameter $\lambda_{\mathbf{q}\nu}$ is proportional to the Fermi-surface average of the electron–phonon matrix element:

$$\lambda_{\mathbf{q}\nu} = 2N(E_F) \frac{\langle |g(\mathbf{n}\mathbf{k}, \mathbf{n}'\mathbf{k}', \mathbf{q}\nu)|^2 \rangle}{\hbar\omega_{\mathbf{q}\nu}} \quad (2)$$

where $N(E_F)$ is the density of states per spin per atom at the Fermi level, $\omega_{\mathbf{q}\nu}$ is the phonon frequency at wavevector $\mathbf{q}$ for branch ν , and the electron–phonon matrix element g is defined as

$$g(\mathbf{n}\mathbf{k}, \mathbf{n}'\mathbf{k}', \mathbf{q}\nu) = \left(\frac{\hbar\Omega_{\text{BZ}}}{2M\omega_{\mathbf{q}\nu}} \right)^{1/2} \left\langle \psi_{\mathbf{n}\mathbf{k}}^0 | \epsilon_{\mathbf{q}\nu} \cdot \frac{\delta V}{\delta \mathbf{R}} | \psi_{\mathbf{n}'\mathbf{k}'}^0 \right\rangle \delta(\mathbf{k} - \mathbf{k}' - \mathbf{q}) \quad (3)$$

where Ω_{BZ} is the volume of the Brillouin zone, M is the atomic

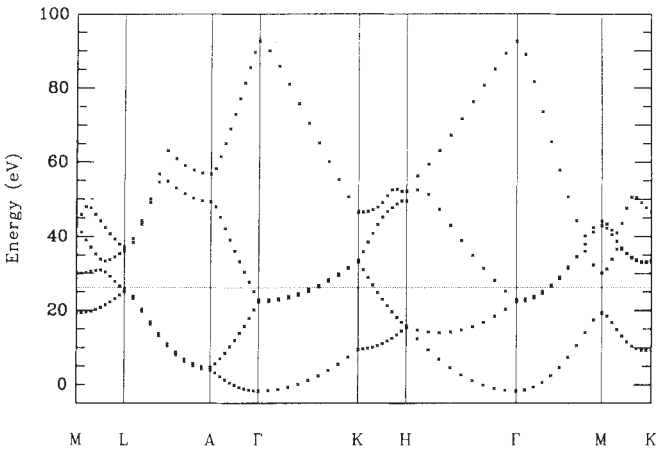


FIG. 1 Band structure of hydrogen in the distorted hexagonal structure presented in the text. The dotted line represents the Fermi level. Fermi-surface nesting may occur for a phonon wavevector of $\mathbf{q}=(2\pi/a)(\frac{1}{3}, 0, 0)$ due to the bands crossing the Fermi surface along the Γ -M line. Γ is at the centre of the Brillouin zone (BZ) with M at the centre of the rectangular face and K on the edge. A, L, and H are on the hexagonal face of the BZ directly above Γ , M, and K, respectively.

mass, $\epsilon_{\mathbf{q}\nu}$, the phonon polarization vector of unit length, $\psi_{n'\mathbf{k}}$ and $\psi_{n\mathbf{k}}$ are Bloch wavefunctions having band indices n' and n and wavevectors $\mathbf{k}'$ and $\mathbf{k}$ and $\delta V/\delta \mathbf{R}$ is the change in the self-consistent crystal potential caused by the phonon distortion. The parameter $\lambda_{\mathbf{q}\nu}$ is related to the experimentally accessible phonon linewidth, $\gamma_{\mathbf{q}\nu}$, by

$$\gamma_{\mathbf{q}\nu} = \pi N(E_F) \hbar \omega_{\mathbf{q}\nu}^2 \lambda_{\mathbf{q}\nu} \tag{4}$$

The phonon frequencies are calculated from the difference in total energy between the undistorted and distorted structures. In our calculation, the change in the self-consistent crystal potential which enters into g is replaced by

$$\epsilon_{\mathbf{q}\nu} \cdot \frac{\delta V}{\delta \mathbf{R}} = \frac{V_{\mathbf{q}\nu} - V_0}{\bar{u}_{\mathbf{q}\nu}} \tag{5}$$

where $\bar{u}_{\mathbf{q}\nu}$ is the r.m.s. phonon amplitude, and V_0 and $V_{\mathbf{q}\nu}$ are the self-consistent potentials found for the undistorted and distorted crystals, respectively.

Within the supercell method, the phonon wavevector $\mathbf{q}$ must be commensurate with the original lattice, that is, $n\mathbf{q} = \mathbf{G}$, where $\mathbf{G}$ is a reciprocal lattice vector of the undistorted crystal. There-

fore, in the supercell, $\mathbf{q}$ is a reciprocal lattice vector and $\mathbf{k}'+\mathbf{q}$ maps back to $\mathbf{k}$, eliminating the δ function in equation (3). The δ functions used in the Fermi-surface average are gaussian broadened to ensure numerical convergence.

The calculations were done at a unit-cell volume slightly smaller than the predicted transition volume for the molecular $\rightarrow$ primitive hexagonal phase transition⁶. The lattice constant a used was 1.38 Å with a c/a ratio of 0.60, corresponding to a Wigner-Seitz radius of 1.30 a.u. For this value of the lattice constant, the calculated pressure is $p \sim 400$ GPa. The phonon frequencies are calculated for several wavevectors and an imaginary harmonic frequency is found for the transverse mode of wavevector $\mathbf{q}=(2\pi/3c)(0,0,1)$. This implies an instability towards a tripling of the unit cell. Indeed, when a larger unit cell with three H atoms and a c/a ratio of 1.80 is considered, small distortions are found in the structure, and the equilibrium positions of the atoms become $\tau=(0,0,0), \pm(0.078,0.078,1.021/3)$ in lattice coordinates. All phonon frequencies calculated for this new structure are real and positive, indicating a stable structure. Figure 1 shows the band structure for the distorted lattice. The energy bands are close to free-electron bands and it is clear that hydrogen in this structure is still metallic. Table 1 lists the contributions to λ from wavevectors along three directions: [100], [001], and [101]. The zone-centre optical phonon ($\mathbf{q}=0$) is also calculated to estimate the contribution of the optical modes to λ . A Brillouin-zone average is performed by taking a spherical average along each direction, averaging the results, and then adding twice the contribution from the zone-centre optical phonons to account for the optical modes.

The value of the electron-phonon interaction parameter λ , obtained from averaging the contributions of the phonon modes shown in Table 1, is $\lambda = 1.5$ and the average value of the phonon frequency $\langle \omega \rangle = 2.4 \times 10^{14}$ rad s⁻¹, corresponding to a temperature $\hbar \langle \omega \rangle / k_B = 1,830$ K. The phonon modes along the Γ -L line within the Brillouin zone were not included due to the difficulty in specifying the normal modes. We estimate the Coulomb parameter μ from free-electron calculations using two models for the static dielectric function and find that Fermi-Thomas and Lindhard screening predict $\mu = 0.187$ and $\mu = 0.196$ respectively²⁰. The renormalized Coulomb repulsion μ^* is then $\mu^* = 0.096$ and $\mu^* = 0.098$, respectively. We therefore use $\mu^* = 0.10$ in our calculations of T_c . Using these values, the McMillan equation (equation (1)) predicts a superconducting transition temperature $T_c = 210$ K, whereas the numerical Eliashberg calculation yields $T_c = 230$ K.

TABLE 1 Electron-phonon parameters and phonon frequencies

$\mathbf{q}, \nu$	$\lambda_{\mathbf{q}\nu}$	$\alpha=1$	$\alpha=\frac{2}{3}$
		$\omega_{\alpha\mathbf{q}\nu}$ (10^{12} rad s ⁻¹)	$\omega_{\alpha\mathbf{q}\nu}$ (10^{12} rad s ⁻¹)
$\alpha(0,0,\frac{1}{2})_L$	0.26	443	0.12
$\alpha(0,0,\frac{1}{2})_{T_X}$	0.47	117	0.40
$\alpha(0,0,\frac{1}{2})_{T_Y}$	0.15	73	0.10
$\alpha(\frac{1}{2},0,0)_L$	0.50	471	0.13
$\alpha(\frac{1}{2},0,0)_{T_Y}$	0.26	197	1.5
$\alpha(\frac{1}{2},0,0)_{T_Z}$	0.15	62	1.05
$\alpha(\frac{1}{2},0,\frac{1}{2})_X$	0.25	400	—
$\alpha(\frac{1}{2},0,\frac{1}{2})_Y$	0.60	170	—
$\alpha(\frac{1}{2},0,\frac{1}{2})_Z$	0.38	455	—
$(0,0,0)_X$	0.02	355	—
$(0,0,0)_Y$	0.07	51	—
$(0,0,0)_Z$	0.10	526	—

The electron-phonon parameter $\lambda_{\mathbf{q}\nu}$ and the phonon frequency $\omega_{\mathbf{q}\nu}$ are shown for several phonons at the Brillouin zone edge ($\alpha=1$) and at two-thirds of the zone-edge wavevector ($\alpha=\frac{2}{3}$). The zone-centre ($\mathbf{q}=0$) optical mode is also shown. The phonon polarization is indicated by L for longitudinal, T for transverse, and X, Y, Z for the three directions in space. These results were calculated for a lattice constant $a=1.38$ Å and a c/a ratio of 1.800.

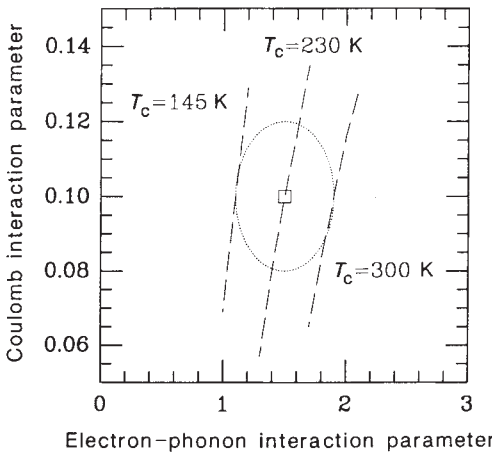


FIG. 2 Calculation of T_c for hydrogen. The dotted line encloses the range of values of the parameters λ and μ^* and the square represents the most likely value. The dashed lines are T_c isotherms calculated from the McMillan equation ($\lambda \leq 1.2$) and numerically using the Eliashberg strong-coupling theory ($\lambda \geq 1.3$).

Averaging λ_q over the Brillouin zone to obtain an estimate of λ uses only a small number of wavevectors (those in Table 1) because the use of a large number of points is computationally expensive within the supercell approach. The sparse sampling of points means that the value of λ reported is only an approximation to the actual value of λ . This is the most serious limitation to the accuracy of our calculation. Because the contributions λ in Table 1 vary over an order of magnitude, it is important to investigate the origin of their variation. In particular, the $\mathbf{q} = (2\pi/a)(\frac{1}{3}, 0, 0)$ phonon is responsible for an exceptionally large contribution to λ . If we remove the $\mathbf{q} = (2\pi/a)(\frac{1}{3}, 0, 0)$ modes from the average, we find that $\lambda = 1.25$ and $T_c = 185$ K. Removal of this one wavevector from the average thus causes nearly a 20% change in the transition temperature, T_c . The band structure presented in Fig. 1 shows two bands crossing the Fermi level at a wavevector $\mathbf{k} = (2\pi/a)(\frac{1}{6}, 0, 0)$. Fermi-surface nesting could,

therefore, explain the overall large value of λ found for these modes. The disparity between contributions to λ from the different polarizations of this mode can be accounted for by the softness of the transversely polarized phonons relative to the longitudinally polarized phonons.

Because T_c depends exponentially on λ and μ^* , a slight change in either parameter can drastically affect T_c . We estimate the range of possible T_c by considering the possible ranges of both λ and μ^* . The uncertainties in μ^* and λ are assumed to be 0.02 and 0.40, respectively. Figure 2 shows the range of values that these parameters can assume together with the isotherms of T_c . From these estimates, we find that T_c should lie between 145 and 300 K, with a probable value of 230 K. Whether or not it will prove possible to attain in the laboratory the pressures necessary to test this prediction remains to be seen, but the results of recent experiments¹⁴ are encouraging in this respect. $\square$

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- Wigner E. & Huntington H. B. *J. chem. Phys.* **3**, 764–770 (1935).
- Ashcroft N. W. *Phys. Rev. Lett.* **21**, 1748–1749 (1968).
- Chakravarty S., Rose J. H., Wood D. & Ashcroft N. W. *Phys. Rev.* **B24**, 1624–1635 (1981).
- Min B. I., Jansen H. J. F. & Freeman A. J. *Phys. Rev.* **B30**, 5076–5083 (1984).
- Min B. I., Jansen H. J. F. & Freeman A. J. *Phys. Rev.* **B33**, 6383–6390 (1986).
- Barbee, T. W. III, Garcia A., Cohen M. L. & Martins J. L. *Phys. Rev. Lett.* **62**, 1150–1153 (1989).
- Gupta R. P. & Sinha S. K. in *Superconductivity in d- and f-band Metals* (ed. Douglass, D. H.) 583–592 (Plenum, New York, 1976).
- Switendick A. C. in *Superconductivity in d- and f-band Metals* (ed. Douglass, D. H.) 593–604 (Plenum, New York, 1976).
- Chang K. J. *et al.* *Phys. Rev. Lett.* **54**, 2375–2378 (1985).
- Dacorogna M. M., Chang K. J. & Cohen M. L. *Phys. Rev.* **B32**, 1853–1855 (1985).
- Bardeen J., Cooper L. N. & Schrieffer J. R. *Phys. Rev.* **106**, 162–164 (1957).
- Bardeen J., Cooper L. N. & Schrieffer J. R. *Phys. Rev.* **108**, 1175–1204 (1957).

- Eliashberg G. M. *Soviet. Phys. JETP* **11**, 696–702 (1960).
- Mao H. K. & Hemley R. J. *Science* **244**, 1462–1465 (1989).
- Cohen M. L. & Anderson P. W. in *Superconductivity in d- and f-band Metals* (ed. Douglass, D. H.) 17–27 (American Institute of Physics, New York, 1972).
- Lam P. K., Dacorogna M. M. & Cohen M. L. *Phys. Rev.* **B34**, 5065–5069 (1986).
- McMillan W. L. *Phys. Rev.* **167**, 331–344 (1968).
- Cohen M. L. *Physica Scripta* **T1**, 5–10 (1982).
- Allen P. B. & Dynes R. C. *Phys. Rev.* **B12**, 905–922 (1975).
- Allender D., Bray J. & Bardeen J. *Phys. Rev.* **B7**, 1020–1029 (1973).

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Carbonaceous aerosols from different tropical biomass burning sources

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FOLLOWING a repetitive pattern, biomass burning affects the intertropical belt on a continental scale during the dry season¹. The importance of these anthropogenic activities with regard to carbonaceous-component emissions into the global atmosphere is now well recognized^{2–4}. It has been suggested that large injections of black carbon aerosols from the Tropics are of potential importance for the radiative and chemical balance of the troposphere^{5–10}. Studies on carbonaceous aerosols have indicated that, on an annual basis, the intensity of the emissions from tropical biomass burning could compare with that of emissions from fossil-fuel burning in industrial countries^{7,8}. Also, results from combustion chamber experiments have determined the important range of the emission factor for both the organic and the black carbon components of the aerosol^{11–16}. Following on from our earlier studies on total atmospheric particulate carbon (C_t) and isotopic composition ($\delta^{13}C$) (ref. 2), we now present new data on the black carbon content (C_b) of atmospheric particles sampled during the biomass-burning season in the wooden savannah of the Ivory Coast. The C_b/C_t ratio is generally lower than expected and highly variable. This variability indicates that there are drastic changes in source apportionment, which from our isotope studies may be ascribed to the variety of vegetation fuel and also to the mode of combustion. Therefore the C_b/C_t ratio can potentially discriminate biomass-burning emissions from different tropical ecosystems.

We sampled atmospheric aerosols at Lamto during the dry season (January–May) of 1980. As a result of the extreme

southward location of the ITCZ (Inter-tropical convergence zone), there is no prevailing wind direction at this site, so the atmosphere is fed by sources that are geographically very different. The aerosol sampled during this period is therefore likely to integrate various local and distant biomass-burning emissions². The particles were collected on pre-cleaned glass-fibre filters, 5 m above ground level. Mean sampling duration was 1 day and mean filtered air volume was 130 m³. The analytical techniques have been described^{2,7,17}. Before analysis, samples were always decarbonated². Total carbon (C_t), black carbon (C_b) and the carbon isotopic composition ($\delta^{13}C$) were determined from aliquots of the same filter. Black carbon (soot) was analysed by coulometric titration after thermal removal of the organic component during a low-temperature precombustion step¹⁷ and the organic-carbon content (C_o) calculated as $C_o = C_t - C_b$.

Figure 1 presents results for 35 individual samples. Black carbon is present in each sample but most of the atmospheric

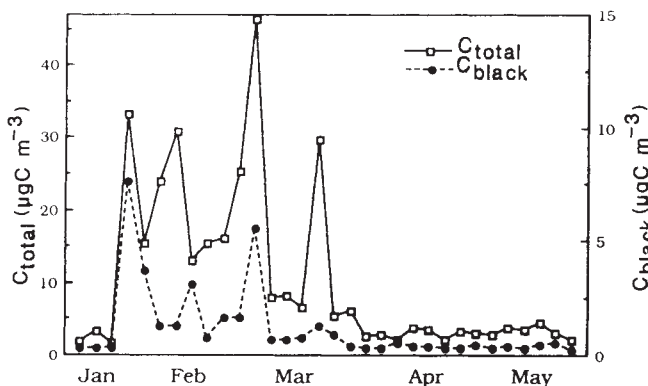


FIG. 1 Black and total carbon contents of aerosols collected during the dry season of 1980 at Lamto (Ivory Coast). Black carbon is defined as the dark component of combustion-derived carbonaceous aerosols.

particulate carbon is organic. There are two distinct sampling periods in the experiment: during the first part (January–March), both total and black carbon concentrations are very high and variable; conversely, during the second period (April–May), carbon loads are rather low and regular. These observations could be related to the occurrence and intensity of the biomass-burning episodes. The first aerosol samples were primarily influenced by large-scale bushfires, whereas during collection of the later samples, the local grounds were bare and there was no significant biomass burning in the region². Although carbon concentrations are very variable throughout the experiment, the mean black and total carbon concentration values (0.7 and 6.2 $\mu\text{g C m}^{-3}$ respectively) for the whole data set are remarkably similar to those found by Andreae *et al.*¹⁰ in the boundary layer above the Amazonian forest during the dry-season ABLE 2 experiment. Also, both black and total atmospheric carbon loads are comparable with those found in temperate urban regions and thus point to the importance of biomass-burning emissions in the area (Table 1).

The concentrations of total and black carbon peak simultaneously (Fig. 1), but differences in aerosol composition are underlined by the variability of the C_b/C_t ratio, which ranges from 4 to 24% (Fig. 2). The value of this ratio is not related to the aerosol carbon load ($r=0.14$) and is thus probably not influenced by nearby fires. Mean C_b/C_t values ($\sim 11\%$) for the two periods of the experiment are similar and comparable to the mean value found in haze layers during the ABLE 2 experiment ($C_b/C_t \sim 13\%$) (ref. 10). This low value may be considered as a characteristic of the mean tropical aerosol and stresses the importance of the emissions of pure organic particulates during biomass-burning processes. Some C_b/C_t values found here for individual samples (4–5%) are the lowest ever reported; an explanation could lie in the exceptional dominance of biomass-burning sources, a situation which never appears elsewhere and particularly not in temperate regions. Furthermore, comparison with data from the Paris region ($C_b/C_t = 22\%$) (ref. 18) indicates that aerosols derived from industrial combustion and biomass-burning may be distinguished by their different C_b/C_t ratios.

There are several explanations possible for these important fluctuations of the C_b/C_t ratio. First, the C_b/C_t ratio could primarily reflect the relative importance in source apportionment of biomass burning and natural (that is, terrigenous aerosols and vegetation emissions) inputs; aerosols with a major natural component would be enriched in organic matter, and under such conditions their C_b/C_t ratio would be low. But the lowest C_b/C_t ratios (4–6%) are found during the fire season and are always associated with high total carbon concentrations ($C_t >$

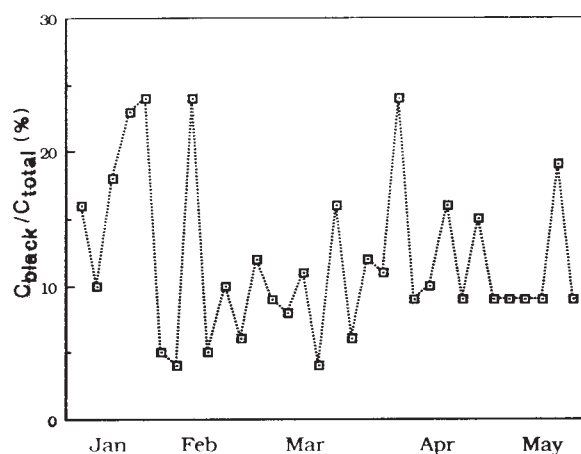


FIG. 2 Variability of the black carbon to total carbon ratio of the dry-season aerosol at Lamto.

15 $\mu\text{g C m}^{-3}$); for these aerosols, biomass burning is certainly the main particulate-carbon source and other major sources can be excluded. Consequently, the diversity of the biomass-burning conditions is likely to control the relative abundance of the different carbonaceous components, and thus the variability of the C_b/C_t ratio, in our set of aerosol samples.

Laboratory experiments have shown that different parameters can account for sizeable changes in emission factors of organic and black carbon aerosols during the vegetation combustion processes. Differences may be related to the variable chemical composition of the plant tissues and thus to the nature of the plant itself¹²; as an example, lignin promotes more black carbon formation than holocellulose¹⁹. But the parameter that has the greatest effect on particulate emissions is the quality of the combustion (smouldering versus flaming and fire intensity). The nature of the burning process is closely related to the O_2 supply and to the size of the burning vegetation fragments¹³, so smouldering fires which are at a rather low temperature and poorly ventilated are, on average, more efficient emitters of atmospheric particles than flaming processes. The particles emitted by such fires are primarily made up of tan organic droplets and have a very low black carbon content¹³. From these considerations, the changes in the C_b/C_t ratio of carbonaceous particles emitted into the atmosphere during biomass burning could be used to trace changes in vegetation fuel or in the nature of the combustion process.

In tropical areas, biomass burning may affect either forested areas or savannahs^{1,2,20}. Savannah bushfires during the dry

TABLE 1 Comparison of the mean carbon concentration values obtained for aerosols sampled at Lamto (Ivory Coast) during the dry season and in the Paris region

		C_{total} ($\mu\text{g C m}^{-3}$)	C_{black} ($\mu\text{g C m}^{-3}$)	C_b/C_t (%)
Lamto				
Fire season				
($n=16$)	a.m.	17.4 (13.0)	1.9 (2.1)	12 (7)
	g.m.	11.9 (2.7)	1.2 (2.7)	10 (1.9)
Transition season				
($n=16$)	a.m.	3.3 (1.2)	0.4 (0.2)	12 (5)
	g.m.	3.2 (1.4)	0.4 (1.4)	11 (1.4)
Whole experiment				
	a.m.	10.4 (11.5)	1.2 (1.7)	12 (6)
	g.m.	6.2 (2.7)	0.7 (2.6)	10.5 (1.7)
Paris region*				
($n=54$)	a.m.	9.6 (5.5)	2.6 (1.3)	22 (4)
	g.m.	8.2 (1.8)	1.8 (1.8)	22 (1.2)

a.m., Arithmetic mean; g.m., geometric mean; (), one standard deviation.

* Data from Brémond *et al.* (ref. 18).

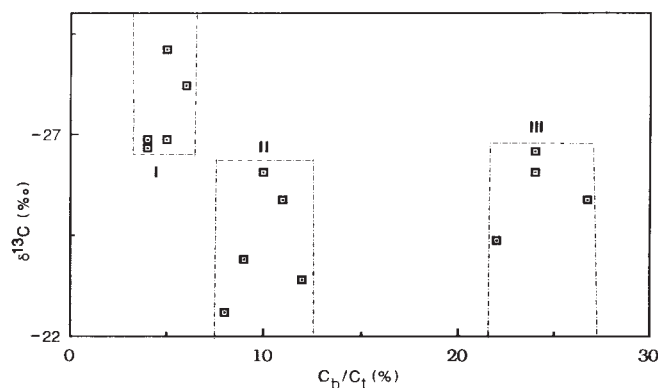


FIG. 3 Isotopic composition ($\delta^{13}\text{C}$) and C_b/C_t ratio of tropical biomass-burning aerosols. All samples with $C_t > 7 \mu\text{g C m}^{-3}$ are considered to originate from a dominant biomass-burning source and selected for this set of data.

season are primarily large-scale fires of long duration^{1,21}. These biomass-burning processes are likely to be fast high-intensity fires and to exhibit rather flaming phases whereas forest fires would present more smouldering phases. Thus, if our explanations are correct, biomass-burning emissions from these two distinct tropical ecosystems would be distinguishable on the basis of their particulate carbonaceous emissions and, more specifically, the contribution of the black carbon component.

Cachier *et al.* (1985)² have been able to differentiate savannah and forest emissions in tropical source regions by $\delta^{13}\text{C}$ measurement of the aerosol. This is because the savannah plants that burn are mostly C4 grasses and are isotopically different ($\delta^{13}\text{C}_{\text{mean}} = -12\%$) from C3 plants ($\delta^{13}\text{C}_{\text{mean}} = -26\%$). Although biomass burning is accompanied by isotope separation, the isotope difference between C4 and C3 plants can be traced in the tropical combustion aerosols ($\delta^{13}\text{C}$ range for the combustion aerosol is: -20 to -24% for savannah grasses and -25 to -30% for trees). We have compared the nature of the different biomass-burning emissions using $\delta^{13}\text{C}$ and C_b/C_t as tracers and samples for which biomass burning was the dominant source of aerosols ($\text{C}_t > 7 \mu\text{g C m}^{-3}$).

These samples separate into three distinct groups (Fig. 3) from which we can gain information about the different biomass-burning situations. The most striking feature of the data is that, for each group, the C_b/C_t range is very narrow and reflects a characteristic combustion process. Another feature is the isotopic composition, which confines to the lowest C_b/C_t group (I) all samples with a $\delta^{13}\text{C} < -26\%$. A possible explanation is that aerosol samples in this group originate exclusively from forest fires, as indicated by the low $\delta^{13}\text{C}$ of the particles², the very low C_b/C_t ratios for these aerosols being consistent with such an origin. On the basis of the $\delta^{13}\text{C}$ values, aerosol samples from groups (II) and (III) probably originate from savannah fires. The separation into two groups of the C_b/C_t values suggests that there are two very different burning patterns in savannahs. During this season different types of biomass-burning process may be occurring: large-scale fires (slash and burn) or limited fires (backfires), but the reasons for this distinction are not yet understood.

So, in tropical regions the different biomass-burning sources and processes generate atmospheric carbonaceous particles which can be distinguished on the basis of their C_b/C_t ratio; this parameter allows us to differentiate between forest and savannah fires. If it is assumed that the carbonaceous particulate material generated during such high-temperature emission processes does not undergo significant physical or chemical transformation then the C_b/C_t ratio is a potential tracer of the aerosol outside the emission region for large-scale biomass-burning events. Shifting of processes²⁰⁻²³ in the inter-tropical latitudinal belt throughout the year or from one year to another could possibly be detected by this tracer. □

20. Woodwell, G. M. *et al. Science* **222**, 1081-1086 (1983).

21. Delmas, R. *Geophys. Res. Lett.* **7**, 761-764 (1982).

22. Booth, W. *Science* **243**, 1428-1429 (1989).

23. Malingreau, J. P. & Tucker, C. J. *La Recherche* **185**, 181-188 (1987).

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Coralline barium records temporal variability in equatorial Pacific upwelling

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LATTICE-BOUND cadmium in scleractinian corals has been shown to be a sensitive tracer of historical changes in the nutrient content of surface waters^{1,2}. Barium also substitutes into the lattice of aragonite reef-building corals because there is solid solution between orthorhombic BaCO_3 (witherite) and CaCO_3 (aragonite)³. It is expected that the substitution should be proportional to the Ba content of sea water, which increases from low values in warm surface waters to higher values in cold deep waters. Here we present a high-resolution coralline Ba record from the Galapagos Islands spanning the period 1965-1978. Coralline Ba/Ca tracks historical sea surface temperatures, reflecting the vertical displacement of warm nutrient-poor surface waters by cold, nutrient-rich source waters. Differences between coralline Ba and Cd records may be due to preferential uptake of Cd by phytoplankton during times of lower surface nutrients.

We have recovered a Ba/Ca record from a cored sample of the reef-building coral *Pavona clavus* collected at Punta Pitt on San Cristobal Island, Galapagos Islands⁴ (Fig. 1; Table 1). The coral was sampled at approximate three-month increments over the period of the record 1965-1978. Coral samples were cut into four divisions of a single year of growth; annual band widths averaged ~ 11 mm. Narrow high-density bands were used as marker points for yearly increments. Corals were cleaned by a series of oxidizing and reducing steps designed for Pb and Cd assay⁵, elements which are considerably more prone to contamination than Ba. After dissolution in Vycor-distilled 2M HNO_3 , the samples were spiked with a known quantity of ^{135}Ba for determination by isotope dilution. Measurement of the $^{135}\text{Ba}/^{138}\text{Ba}$ ratio was made on a VG PlasmaQuad inductively coupled plasma/mass spectrometer (ICP/MS). Accuracy was controlled by calibration of isotope dilution determinations against a gravimetric standard. The barium detection limit is 0.1 nM; for coral samples the ratio of signal-to-noise was always greater than 100:1. Typical sample size corresponded to 1 mg of dissolved coral. Ca was measured by flame atomic absorption. Replicates of Ba/Ca ratios of individual coral samples were reproducible to 2% on different days; to optimize the internal precision of the data set, however, we ran the 1965-1978 record on a single day. On that day, eight replicate determinations of 0.5 ml of a consistency standard containing 95.7 nM Ba and 18.2 mM Ca yielded a reproducibility (1 standard deviation) of 0.9% for Ba, 1.0% for Ca and 1.0% for Ba/Ca over the course of this run. Samples for Sr were spiked with ^{87}Sr and concentrations determined by the isotope dilution measurement of the $^{87}\text{Sr}/^{88}\text{Sr}$ ratio on the ICP/MS. Precision of the Sr/Ca ratios is $\sim 1\%$.

The Ba/Ca ratio measured on the Galapagos coral ranges from 4.1 to 5.1 $\mu\text{mol mol}^{-1}$; this natural variation of 1 μmol

Received 17 April; accepted 13 June 1989.

1. Seiler, W. & Crutzen, P. J. *Climatic Change* **2**, 207-247 (1980).

2. Cachier, H., Buat-Ménard, P., Fontugne, M. & Rancher, J. *J. Atmos. Chem.* **3**, 469-489 (1985).

3. Harriss, R. C. *et al. J. geophys. Res.* **93**, 1351-1360 (1988).

4. Greenberg, J. P., Zimmerman, P. R., Heidt, L. & Pollock, W. *J. geophys. Res.* **89**, 1350-1354 (1984).

5. Andree, M. O. *Science* **220**, 1148-1151 (1983).

6. Turco, R. P., Toon, O. B., Whitten, R. C., Pollack, J. B. & Hamill, P. in *Precipitation, Scavenging, Dry Deposition and Resuspension Vol. 2* (eds Pruppacher, H. *et al.*) 1337-1351 (New York, Elsevier, 1982).

7. Cachier, H., Buat-Ménard, P., Fontugne, M. & Chesselet, R. *Tellus* **38B**, 161-177 (1986).

8. Cachier, H. thesis, Univ. Paris (1987).

9. Suman, D. *J. Atmos. Chem.* **6**, 21-34 (1988).

10. Andree, M. O. *et al. J. geophys. Res.* **93**, 1509-1527 (1988).

11. Crutzen, P. J., Galbally, I. E. & Brühl, C. *Climatic Change* **6**, 323-364 (1984).

12. Muhlbaier, J. L. & Williams, R. L. in *Particulate Carbon Atmospheric Life Cycle* (eds Wolff, G. T. & Klimisch, R. L.), 185-198 (New York, Plenum, 1982).

13. Patterson, E. M., McMahon, C. K. & Ward, D. E. *Geophys. Res. Lett.* **13**, 129-132 (1986).

14. Cooper, J. A. *J. Air Pollut. Control Assoc.* **30**, 855-861 (1980).

15. Dod, R. L., Brown, N. J., Mowrer, F. W., Novakov, T. & Williamson, R. B. *Aerosol Sci. Technol.* **10**, 20-27 (1989).

16. Rau, J. A. *Aerosol Sci. Technol.* **10**, 181-192 (1989).

17. Cachier, H., Brémond, M. P. & Buat-Ménard, P. *Tellus* **41B**, 379-390 (1989).

18. Brémond, M. P., Cachier, H. & Buat-Ménard, P. *Environ. Tech. Lett.* **10**, 339-346 (1989).

19. Tillman, D. A. in *Woods as an Energy Resource* (Academic, New York, 1978).

mol^{-1} is ~ 10 -times greater than the inter-run precision of $\pm 0.1 \mu\text{mol mol}^{-1}$ and 20-times greater than the intra-run precision of $\pm 0.05 \mu\text{mol mol}^{-1}$. Our values generally agree with recent determinations of Ba in corals^{2,6-8}. Some earlier studies give values up to 10-times higher than our values^{9,10}; this could be due to insufficient cleaning of these coral samples to remove residual non-lattice-bound Ba, such as that associated with organic tissues, which can contain orders of magnitude more Ba than is present in the coral skeleton⁸.

Variability in recorded Ba values of *P. clavus* at Punta Pitt in the Galapagos Islands results from changes in the intensity of upwelling of cold, nutrient-rich source waters to the surface ocean. Shoaling of the thermocline in the eastern equatorial

Pacific allows cold, deep Ba-rich waters to mix into the surface layer. Upwelling variability is documented for the eastern equatorial Pacific in the form of historical sea surface temperature (SST) records¹¹. An SST record, available from Academy Bay on Santa Cruz Island¹², shows that although Academy Bay is $\sim 1-2^\circ\text{C}$ warmer than Punta Pitt during normal climatic conditions, records of temperature variability at the two sites are similar⁴. Comparison of the Academy Bay SST record with our coral record of Ba/Ca indicates coherent variability between these two records (Fig. 1a). Cold peaks in the temperature record line up with relative maxima in the Ba record, and warm peaks in the temperature record align with relative minima. This correspondence is, however, not perfect, the largest discrepancies arising in the precise temporal location of the peaks (1965-66; 1968-69; 1973-74). We attribute these differences primarily to our inability to sample the coral perfectly, as sectioning of single-year growth increments into four even sections does not guarantee that each section actually represents three months of growth. In addition, there is always some

TABLE 1 Ba/Ca and Sr/Ca values for the Punta Pitt section

Year	Quarter	Ba/Ca ($\mu\text{mol mol}^{-1}$)	Sr/Ca (mmol mol^{-1})
1965-	1	4.36	9.33
	2	4.60	9.46
	3	4.44	9.42
	4	4.22	9.21
1966-	1	4.34	9.34
	2	4.94	9.61
	3	4.79	9.52
	4	4.54	9.49
1967-	1	4.44	9.52
	2	4.94	9.60
	3	4.85	9.65
	4	4.78	9.62
1968-		4.66	9.61
	2	4.61	9.59
	3	4.16	9.24
	4	4.34	9.22
1969-	1	4.41	9.44
	2	4.55	9.54
	3	4.84	9.54
	4	4.46	9.41
1970-	1	4.43	9.26
	2	4.77	9.49
	3	4.85	9.70
	4	4.88	9.80
1971-	1	4.68	9.55
	2	4.53	9.27
	3	4.75	9.63
	4	4.66	9.11
1972-	1	4.41	9.13
	2	4.29	9.39
	3	4.51	9.44
	4	4.36	9.48
1973-	1	4.31	9.44
	2	4.79	9.86
	3	4.92	9.74
	4	4.39	8.93
1974-	1	4.66	9.57
	2	4.75	9.46
	3	4.88	9.64
	4	4.95	9.71
1975-	1	4.67	9.55
	2	4.78	9.60
	3	5.05	9.84
	4	4.83	9.54
1976-	1	4.45	9.55
	2	4.42	9.66
	3	4.56	9.48
	4	4.55	9.52
1977-	1	4.34	9.56
	2	4.62	9.55
	3	4.89	9.75
	4	4.77	9.66
1978-	1	4.39	9.32
	2	4.63	9.33
	3	5.02	9.35

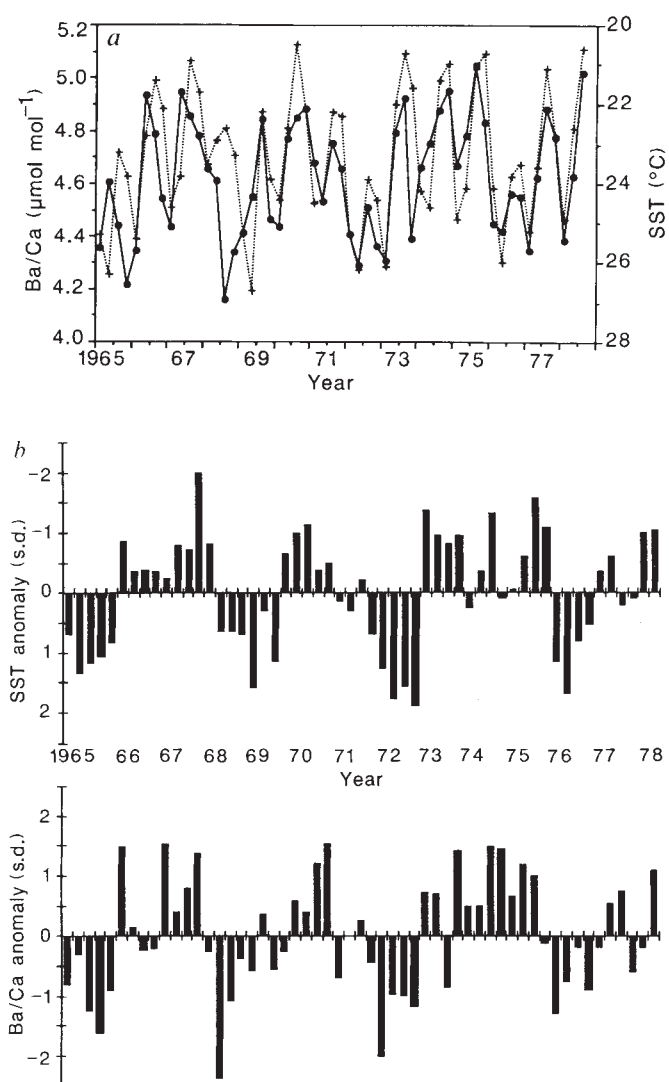


FIG. 1 a, Ba/Ca (solid line) measured in quarter-annual bands of the coral *P. clavus* (Punta Pitt, San Cristobal Island, Galapagos Islands) compared with sea surface temperature (SST) (broken line) recorded at Academy Bay (Santa Cruz Island, Galapagos Islands)¹² over the time period 1965-1978. b, Quarterly anomalies of Ba/Ca in the Punta Pitt coral record compared with quarterly anomalies in SST at Academy Bay over the time period 1965-1978. Anomalies were calculated by normalizing the difference from the mean for each quarter to the standard deviation of the mean for each quarter.

difficulty in assigning a single year of growth to a band width. These sectioning limitations will also affect the magnitude of the Ba peaks that we measure. Seasonal averaging of the signals truncates the extremities of the record, but this bias is alleviated by comparing the Ba record with an SST record for which points are averaged over three months. These difficulties should, however, be kept in mind when comparing the two records.

Relatively cool temperatures in the eastern equatorial Pacific are maintained in part by the entrainment of colder, upper-thermocline waters which lie close to the base of a very shallow mixed layer¹³. Depression of the usually shallow thermocline resulting from atmospheric forcing by the trade winds¹⁴ causes large positive excursions in temperature which are classified as El Niño/Southern Oscillation (ENSO) events. Such events show up best on SST anomaly plots, such as Fig. 1b, which compares SST anomalies with Ba anomalies. The El Niño events of 1965, 1969, 1972–73 and 1976 (ref. 15) are accompanied by positive excursions in temperature and negative excursions in Ba/Ca. Depression of the thermocline during El Niño events reduces the entrainment of cold, Ba-rich waters, resulting in the coincidence of positive temperature anomalies and negative Ba/Ca anomalies.

As both Cd and Ba are enriched in the upwelled cold-source waters, they show a strong correspondence in the coral records (Fig. 2). Because the Cd-depth gradient is much greater than the gradient for Ba, however, Cd is more sensitive to changes in the vertical restructuring of source waters—compare the four-fold variation in Cd/Ca ratios with the 20% variation in Ba/Ca ratios. Another strong difference is that Ba seems to be more responsive to weak upwelling events (Fig. 2, 1969, 1971, 1972, 1977 and 1978). Fundamental differences in the oceanographic controls on Cd and Ba may account for this behaviour. Cd is more bio-active than Ba in surface waters and hence stripped out more rapidly by biological activity. We speculate that during periods of lower upwelling, Cd may be scavenged almost as fast as it is supplied; only during periods of more intense upwelling does supply exceed removal.

Conversion of the coral Ba values to seawater Ba contents requires estimation of a distribution coefficient, $D = (\text{Ba/Ca})_{\text{coral}} / (\text{Ba/Ca})_{\text{seawater}}$. We calculate $D_{\text{Ba}} = 1.41 \pm 0.14$ based on the Punta Pitt Ba/Ca data and the Ba content of surface sea water at the nearest GEOSECS station (station 331: 125° W, 5° S)¹⁶. Determination of D_{Ba} is better for corals from areas where variability in surface Ba as a result of upwelling is minimal. We have, therefore, measured Ba/Ca ratios in two species of coral collected from North Rock, off Bermuda Island. Two samples of *Diploria labyrinthiformis* yield a Ba/Ca ratio of 5.21, and two samples of *Montastrea annularis* yield a Ba/Ca ratio of 5.32 ± 0.08 . The Bermuda coral measurements give $D_{\text{Ba}} = 1.27 \pm 0.03$, based on the Ba content of surface water at the nearest GEOSECS station (station 29: 36° N, 47° W)¹⁶. Both estimates agree within error; a more precise determination of D is not possible without simultaneous determination of coral and

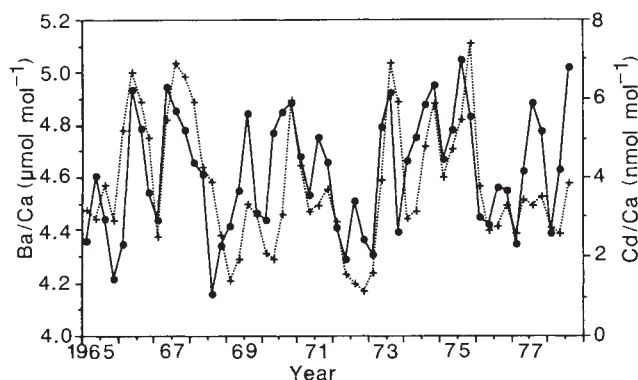


FIG. 2 Comparison of Ba/Ca (solid line) and Cd/Ca (ref. 2) (broken line) in the Punta Pitt coral record over the time period 1965–1978.

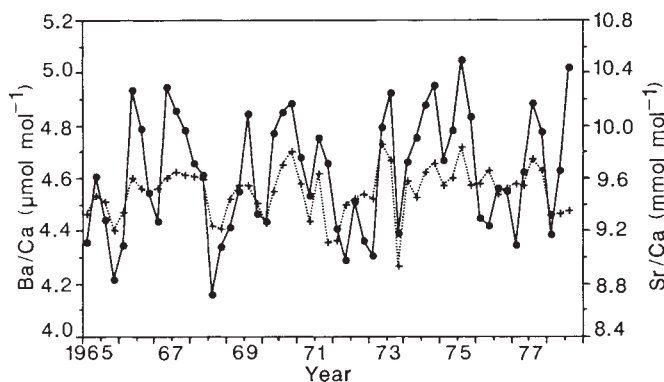


FIG. 3 Comparison of Ba/Ca (solid line) and Sr/Ca (broken line) in the Punta Pitt coral record over the time period 1965–1978. The plot is scaled so that approximately the same amount of variance is shown for each ratio.

sea water. In addition, there could be differences in D_{coral} for the various species, although our measurements of Ba/Ca ratios in the three species studied so far seem to rule out large differences. Corals take up several metals with a distribution coefficient of ~ 1 (refs 2 and 6). The calculated thermodynamic distribution coefficient for Ba in aragonite is 1.3 (ref. 2), so our values agree with the value predicted from thermodynamics.

Converting coralline Ba/Ca at the Punta Pitt site to seawater Ba values using $D_{\text{Ba}} = 1.3$, yields a range of 33 nmol kg^{-1} during the warmest quarters, to 40 nmol kg^{-1} during the coolest quarters. The lower range is typical of values for nutrient-depleted Pacific surface water ($34 \pm 1 \text{ nmol kg}^{-1}$) (ref. 16). Although a lack of data for Ba-depth gradients in the Galapagos region makes an estimation difficult, the upper range can be reconciled with previous estimates of the maximum depth of source waters to the eastern equatorial Pacific, which are in the range $135\text{--}225 \text{ m}^{16-19}$.

A complication in calculating source-water depths from coral Ba/Ca values is the possibility that coral uptake of Ba is influenced by temperature or biologically mediated factors. To test this hypothesis we have recovered a record of chemically similar Sr for the Punta Pitt coral (Fig. 3; Table 1). Unlike Ba, seawater Sr/Ca is essentially conservative in sea water, with a slight upper-ocean depletion of $\sim 1\%$ (ref. 20). Variation in coralline Sr has generally been ascribed to a temperature effect on incorporation, but taxonomy, metabolism and growth rate have also been cited as possible controlling factors^{21,22}. Figure 3 indicates distinct similarities between the Sr/Ca and Ba/Ca records. Although we do not know if this co-variance is caused by a temperature effect on incorporation, the similarity between the two records suggests that some of the Ba signal is due to factors other than variations in the upwelling of source waters with different chemical signatures. The 17% average difference in Ba/Ca between warm and cold quarters is reduced to $\sim 12\%$ when the Ba signal is normalized to Sr rather than Ca, indicating that up to one-third of the Ba/Ca signal could be due to a temperature effect on incorporation. If incorporation of Sr and Ba in this coral is temperature-dependent, then Ba/Sr ratios might be a better indicator of changes in the Ba concentration of surface waters.

In conclusion, Ba/Ca ratios can be a powerful tracer of historical variability in upwelling and entrainment of nutrient-rich deep waters into the surface ocean. Comparison of Ba records with coral records of more bio-active metals like Cd can give an insight into the relative behaviour of the metals in the surface ocean. Finally, long coral records could be used to reconstruct changes in upwelling of cold source waters to pre-historic oceans. □

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- Shen, G. T., Boyle, E. A. & Lea, D. W. *Nature* **328**, 794–796 (1987).
- Shen, G. T. & Sanford, C. L. in *Global Ecological Consequences of the 1982–1983 El Niño* (ed. Glynn, P. W.) (Elsevier, New York, in the press).

3. Speer, J. A. in *Reviews in Mineralogy* (ed. Ribbe, P. H.) **11**, 145–190 (Mineralogical Society of America, Washington, 1983).
4. McConnaughey, T. *Geochim. cosmochim. Acta* **53**, 151–162 (1989).
5. Shen, G. T. & Boyle, E. A. *Chem. Geol.* **67**, 47–62 (1988).
6. Shen, G. T. *Lead and Cadmium Geochemistry of Corals: Reconstruction of Historic Perturbations in the Upper Ocean* (Massachusetts Institute of Technology/Woods Hollow Oceanographic Institute, 1986).
7. Ohde, S., Ohta, N. & Tomura, K. *J. Radioanal. Chem.* **42**, 159–167 (1978).
8. Buddemeier, R. W., Schneider, R. C. & Smith, S. V. in *Proc. 4th Int. Coral Reef Symp.* **2**, 81–85 (1981).
9. Livingston, H. D. & Thompson, G. *Limnol. Oceanogr.* **16**, 786–796 (1971).
10. Flor, T. H. & Moore, W. S. in *Proc. 3rd Int. Coral Reef Symp.* **2**, 555–561 (1977).
11. Rasmusson, E. M. & Carpenter, T. H. *Monthly Weather Rev.* **110**, 354–384 (1982).
12. *Sea Surface Temperature on Santa Cruz Island* (Charles Darwin Research Station, 1988).
13. Wyrki, K. *J. phys. Oceanogr.* **11**, 1205–1214 (1981).
14. Wyrki, K. *J. phys. Oceanogr.* **5**, 572–584 (1975).
15. Rasmusson, E. M. *Oceanus* **27**, 5–12 (1984).
16. Ostlund, H. G., Craig, H., Broecker, W. S. & Spencer, D. W. *GEOSecs Atlantic, Pacific and Indian Ocean Expeditions Vol. 7* (NSF, Washington, DC, 1987).
17. Bryden, H. L. & Brady, E. C. *J. phys. Oceanogr.* **15**, 1255–1273 (1985).
18. Fine, R. A., Peterson, W. H. & Ostlund, H. J. *J. phys. Oceanogr.* **17**, 553–564 (1987).
19. Quay, P. D., Stuiver, M. & Broecker, W. S. *J. mar. Res.* **41**, 769–792 (1983).
20. Brass, G. W. & Turekian, K. K. *Earth planet. Sci. Lett.* **23**, 141–148 (1974).
21. Smith, S. V., Buddemeier, R. W., Redalje, R. C. & Houck, J. E. *Science* **204**, 404–407 (1979).
22. Weber, J. N. *Geochim. cosmochim. Acta* **37**, 2173–2190 (1973).

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In situ collection of diagenetic iron and manganese oxyhydroxides from natural sediments

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IRON and manganese oxyhydroxides are constituents of natural aquatic systems and are thought to play a predominant role in the cycling of toxic trace elements in both marine and fresh-water sediments. Because of their dilution in the sediment matrix (typically 0.1–3.0% dry weight) and because no adequate separation technique was available until now, very little is known about their physico-chemical properties and so progress in the modelling of trace-element partitioning and cycling in aquatic sediments has been limited. We have developed a technique which enables the *in situ* separation and collection of these key constituents by inserting Teflon sheets into lake sediments. The mineralogical composition and surface-adsorption properties of distinct and relatively pure deposits of natural iron and manganese oxyhydroxides can now be studied with conventional instruments and techniques.

The burial of sediments containing iron and manganese oxyhydroxides leads to the reductive dissolution of these compounds below the oxic-anoxic interface. The reduced dissolved iron (II) and manganese (II) thus generated can diffuse upward, be re-oxidized in the oxic layer of the sediments and form new diagenetic Fe(III) and Mn(IV) oxyhydroxides^{1,2}. These diagenetically formed compounds are suspected to be very reactive and to play an important role (through sorption-desorption processes) in the cycling and bioavailability of trace elements such as As, Cd, Cu, Ni, Pb and Zn in aquatic systems^{3–5}. Iron and manganese oxyhydroxides occur in a variety of solid forms,

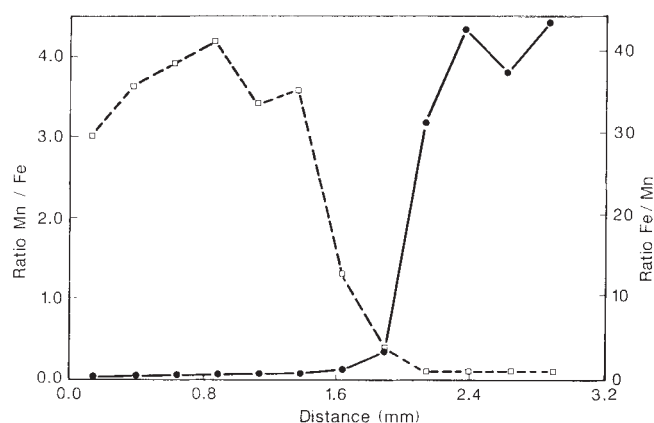


FIG. 1 A transect analysis of the interfacial zone between the iron and manganese layers for Brady Lake using scanning electron microscopy and an EDS microprobe. The Mn/Fe ratios are shown as open squares and the Fe/Mn ratios are shown as closed circles. A 2×2 cm slice of a Teflon collector with both diagenetic Fe and Mn oxyhydroxide deposits was placed in a vacuum chamber and was sputtered with a 500 Å layer of graphite. This sample was analysed in Jeol Model 330 scanning electron microscope equipped with a Kevex 7000 energy dispersive microprobe system. The Fe K α peak intensity includes a minor contribution of the Mn K β peak intensity as the two peaks overlap at 6.45 keV leading to a slight underestimation of the Mn/Fe ratio in the Mn zone.

including both amorphous and crystalline types, each of which may differ in its affinity for trace elements. The precise forms of the oxyhydroxides present in natural sediments have not yet been elucidated. This is because these oxyhydroxides are only minor constituents (typically less than 3% on a dry-weight basis) of bottom sediments; they are thus lost in a complex matrix that prevents the determination of their intrinsic physical and chemical properties (such as adsorption and acidity constants, specific surface area, site density, chemical composition) using classical laboratory techniques. The lack of an appropriate separation technique led researchers to use harsh reagents to dissolve the sediment matrix (silicates and organic matter) to isolate iron oxyhydroxides⁶, but this treatment may be expected to modify the degree of crystallinity of the oxides⁷.

In field work we have observed that natural iron oxyhydroxides are often deposited on plastic or glass surfaces in contact with sediments. This prompted us to develop a method that would allow the *in situ* collection of these diagenetic compounds. In the laboratory, we tested the ability of a variety of materials to collect iron oxyhydroxides. Table 1 indicates that Teflon is the most efficient collector (measured as the amount of iron collected per unit surface area of the material); we note that the ability of the surfaces to collect iron oxyhydroxides is inversely correlated with their reported surface energies⁸. Teflon is not contaminated with trace elements and does not adsorb dissolved trace elements⁹, so it is an ideal surface for studying the *in situ* adsorption of trace elements on diagenetic oxyhydroxides.

A diver vertically inserted Teflon sheets (8×15 cm, 1.5-mm thick) which were left for ten weeks in the sediments of three Canadian Shield fresh-water lakes of varying pH values: Brady

TABLE 1 Mean deposition of iron oxyhydroxides on various materials

Materials	Glass	Plexiglass	Polystyrene	Polyvinylchloride	Teflon
Fe deposit* ($\mu\text{g cm}^{-2}$)	0.6±0.1	0.4±0.1	1.3±0.2	0.9±0.3	3.6±0.3

Materials were submerged in a buffered solution of Fe(II) which was allowed to oxidize at pH 6.0±0.3. The iron oxyhydroxides deposited on the materials were dissolved in a reducing solution and the iron was analysed by flame atomic absorption spectroscopy (AAS).

* N=3 samples; ±1 uncertainty is one standard deviation

TABLE 2 Trace-element concentrations in iron and manganese deposits obtained for three lakes

Lake	pH*	Fe ⁺ (μg)	Mn ⁺ (μg)	As ⁺ (ng)	Cd ⁺ (ng)	Cu ⁺ (ng)	Ni ⁺ (ng)	Pb ⁺ (ng)	Zn ⁺ (ng)
Brady (Mn layer)	6.8	45	173	25	2.2	34	206	131	1,090
Brady (Fe layer)	6.8	860	51	80	1.5	58	70	326	790
Tantaré (Fe layer)	5.6	652	2.7	120	<1.0	50	130	910	430
Clearwater (Fe layer)	4.7	787	0.3	1,340	3.6	1,700	410	997	420

Oxyhydroxides were dissolved in a reducing solution and analysed by AAS. For Brady Lake, Mn and Fe deposits were manually separated with a scalpel.

* Measured at the sediment-water interface.

† Quantities deposited on two Teflon collectors installed at the same location.

(pH 6.8, 46°04' N, 78°49' W); Tantaré (pH 5.6, 47°04' N, 71°32' W); Clearwater (pH 4.7, 46°22' N, 81°03' W). The pH values were measured at the sediment-water interface¹⁰. We have found that the Teflon surface collected not only iron oxyhydroxides formed *in situ*, but also manganese oxyhydroxides and even sulphides. The blackish deposit of iron sulphide was rapidly oxidized when removed from the sediment, turning light brown within a few hours. An additional advantage of the sampling method is that the various diagenetic products collected on the Teflon sheets are spatially resolved and are deposited in the sequence predicted from thermodynamic and kinetic considerations¹¹. Indeed, given the following equations for the negative of the base-10 logarithm of the electron activity pE

$$\text{pE} = \log \left(\frac{\{\text{amorph. Fe(OH)}_3(\text{s})\}}{\{\text{Fe}^{2+}\}} \right) + 17.1 - 3\text{pH} \quad (1)$$

$$\text{pE} = \frac{1}{2} \log \left(\frac{\{\text{MnO}_2(\text{s})\}}{\{\text{Mn}^{2+}\}} \right) + 21.5 - 2\text{pH} \quad (2)$$

we calculate pE values of 2.4 and 14.3 if we assume a pH value of 6.8, Fe²⁺ and Mn²⁺ activities of 2.0 μm and 0.2 μm, respectively, and activities of unity for the solid phases. Thus, the oxidation of manganese requires a higher pE value than that of iron, and pE is expected to decrease downward within the sediments. Also, at a given pH, the rate of chemical oxidation of Mn(II) is much slower than that of Fe(II), which would also favour the formation of manganese oxyhydroxides closer to the sediment-water interface. In the other two lakes where the pH was lower, there was no evidence of manganese oxyhydroxide formation. This notable absence is readily explained by the very slow kinetics of Mn(II) oxidation for pH values below 6.0 (ref. 11). The manganese measured in these lakes is probably sorbed on iron oxyhydroxides.

An energy-dispersive spectroscopy (EDS) microprobe transect of the interfacial zone between the Fe and the Mn layers in a sample collected in Brady Lake, reveals three distinct zones (Fig. 1): (1) a manganese-rich layer characterized by a Mn/Fe peak intensity ratio of ~3.5; (2) an intermediate transition zone which occurs within a vertical distance in the sediment of 500±100 μm; and (3) an iron-rich layer with an Fe/Mn peak intensity ratio of ~40. The difference of one order of magnitude between the Fe/Mn and Mn/Fe peak intensity ratios suggests that a significant amount of Fe is found in the Mn zone whereas very little Mn is associated with iron oxyhydroxides. This is consistent with thermodynamic and kinetic considerations: the

low pE value at which Fe(II) is oxidized to Fe(III), and subsequently precipitated as iron oxyhydroxides and deposited on the Teflon collector is too low for Mn(II) to be oxidized to Mn(IV) and thus deposited. Iron deposition can, however, extend to the manganese deposition zone depending on the relative rates of Fe(II) oxidation and precipitation and the upward diffusion of this metal. Contamination of the Teflon collector by other major inorganic constituents of sediments such as feldspars, and clays was minimal, as the EDS spectra did not contain any large peaks other than those for Fe and Mn. Small peaks of Ca and Si were observed, however, which appeared to have a random distribution relative to Fe and Mn. In the Mn layer, the Ca and Si peak intensities were about 30 and 10 times smaller, respectively, than the Mn peaks. Similar peak ratios relative to Fe were found in the Fe layer. The relative purity of the oxyhydroxides collected should greatly facilitate the physico-chemical characterization of these solid phases by X-ray diffraction, electron microscopy and Mössbauer spectrometry. Furthermore, changes in the forms and degree of crystallinity of the solids should be readily followed by leaving the collectors in the sediments for various time periods.

The vertical resolution of the Mn and Fe deposits suggested that they can be manually separated for chemical analysis. For the samples from Brady Lake, the quantities of Fe and Mn obtained in each layer (after reductive dissolution with a 0.04 M NH₂OH·HCl in 0.1 M HCl solution which was heated at 60 °C for 6 h in acid-washed Teflon vials) confirmed that the separation can be done easily (Table 2). Results for associated trace elements indicate that Zn and Ni are preferentially retained by Mn oxyhydroxides, whereas As and Cu are associated mostly with iron oxyhydroxides. We believe that this method of isolation and separation of these two diagenetic compounds will make it possible to study their properties, in particular their adsorption characteristics, such as site density (by fast tritium exchange¹²) pH_{zpc} the pH of the charge zero-point and intrinsic acidity constants (by acid-base titration,¹³) specific surface area (by Brunauer-Emmett-Teller adsorption isotherm,¹⁴) adsorption constants (by metal titrations or *in situ* measurements¹⁵⁻²⁰). This collection technique could also be useful to determine the redox state of sorbed species (such as As, Cr, Se, Sn, V) in natural sediments. All this information would be invaluable in verifying whether the adsorption measurements with laboratory-prepared iron and manganese oxyhydroxide phases are applicable to model trace-element behaviour in natural sediments. □

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1. Froelich, P. N. *et al. Geochim. cosmochim. Acta* **43**, 1075-1090 (1979).
2. Aller, R. C. *Adv. Geophys.* **22**, 351-415 (1980).
3. Jenne, E. A. *Adv. Chem. Ser.* **73** (ed. Gould, R. F.) 337-387 (American Chemical Society, Washington, 1968).
4. Li, Y. H. *Geochim. cosmochim. Acta* **45**, 1659-1664 (1981).
5. Lion, L. W., Altmann, R. S. & Leckie, J. O. *Envir. Sci. Technol.* **16**, 660-666 (1982).
6. Kampf, N. & Schwertmann, U. *Clays & Clay Miner.* **30**, 401-408 (1982).
7. Goodman, B. A., Berrow, M. L. & Russell, J. D. *J. Soil Sci.* **39**, 87-98 (1988).
8. Zisman, W. A. *Adv. Chem. Ser.* **43** (ed. Gould, R. F.) 1-51 (American Chemical Society, Washington, 1964).
9. Ashton, A. & Chan, R. *Analyst* **112**, 841-844 (1987).
10. Carignan, R. *Limnol. Oceanogr.* **29**, 667-670 (1984).
11. Stumm, W. & Morgan, J. J. *Aquatic Chemistry*, 2nd edn (Wiley-Interscience, New York, 1981).

12. Yates, D. E. & Healy, T. W. *J. Colloid Interface Sci.* **55**, 9-19 (1976).
13. Huang, C. P. & Stumm, W. *J. Colloid Interface Sci.* **43**, 409-420 (1973).
14. Davis, J. A. & Leckie, J. O. *J. Colloid Interface Sci.* **67**, 90-107 (1978).
15. Balistrieri, L. S. & Murray, J. W. *Geochim. cosmochim. Acta* **47**, 1091-1098 (1983).
16. Millward, G. E. & Moore, R. M. *Water Res.* **16**, 981-985 (1986).
17. Benjamin, M. M., Hayes, K. F. & Leckie, J. O. *J. Wat. Pollut. Control Fed.* **54**, 1472-1481 (1982).
18. Johnson, C. A. *Geochim. cosmochim. Acta* **50**, 2433-2438 (1986).
19. Tessier, A., Rapin, F. & Carignan, R. *Geochim. cosmochim. Acta* **49**, 183-194 (1985).
20. Tessier, A., Carignan, R., Dubreuil, B. & Rapin, F. *Geochim. cosmochim. Acta* (in the press).

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CO₂-enhanced melting of biotite-bearing rocks at deep-crustal pressure-temperature conditions

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THE role of CO₂ in melting of the Earth's crust has been a topic of considerable debate. Experimental studies in quartz-feldspar systems^{1,2} have shown that CO₂ solubility in simple granitic liquids is less than 1 wt% at pressures below 10 kbar and that dilution of H₂O vapour with CO₂ raises the vapour-saturated solidus³, indicating that CO₂ inhibits, rather than promotes, crustal melting. On the other hand, from experiments on the system K₂O-MgO-Al₂O₃-SiO₂-H₂O-CO₂, Wendlandt has inferred significant solubility of CO₂ in crustal melts⁴. Wendlandt observed that biotite-quartz-feldspar assemblages melt to orthopyroxene + liquid at temperatures as low as 740 °C in the presence of CO₂-rich fluids, more than 50 °C lower than equivalent orthopyroxene-producing reactions in the pure-H₂O systems⁵. This surprising result implies a fundamental melt interaction between MgO and CO₂, because neither component alone has significant solubility in granitic liquids near the H₂O-saturated solidus⁶. In view of the potentially important consequences of these results for deep-crustal processes, additional data are needed. Here we report the results of experimental melting studies on synthetic biotite-quartz and biotite-quartz-sanidine assemblages. In general agreement with Wendlandt's results, we find that these assemblages melt in the presence of CO₂-rich vapour below 760 °C at pressures <10 kbar, conditions believed to be encountered in high-grade crustal metamorphism. This opens up the possibility that melting of the deep crust may occur in the presence of CO₂-rich fluids.

Starting materials for the present investigation included phlogopite (KMg₃AlSi₃O₁₀(OH)₂) and sanidine (KAlSi₃O₈), both synthesized hydrothermally from the constituent oxides. Mixtures of phlogopite + quartz (natural) in 2:3 weight ratio and phlogopite + sanidine + quartz (2:2:3) were encapsulated with weighed amounts of oxalic acid dihydrate and anhydrous oxalic acid in platinum tube segments. The CO₂:H₂O ratio of equilibrated vapours was determined by relative weight loss on puncture of the capsule at 25 °C, compared with post-puncture weight loss after heating at 110 °C.

Vapour compositions were controlled by the amounts of volatiles in the charges. One type of run had CO₂ + H₂O equal to 30–50 wt% of the charge; the overwhelming abundance of CO₂ + H₂O in these charges maintained a nearly fixed vapour composition. The other type of run had CO₂ + H₂O equal to 9–12 wt% of the charge; this low abundance allowed changes of vapour composition, depending on whether phlogopite in the starting assemblage grew, dehydrated or participated in a melting reaction, because each of these processes changes the initial CO₂:H₂O ratio of the fluid. Dehydration tends to increase the mole fraction of H₂O in the fluid, whereas melting tends to decrease the mole fraction of H₂O in the fluid.

Internally heated argon pressure vessels⁷ were used for runs <6 kbar. Runs at pressures >6 kbar were made using a piston-cylinder apparatus having a NaCl medium of 0.75-in. diameter. Temperatures were measured with Cr-Al thermocouples, and are considered accurate to within 10 °C. Quenched charges were examined by the polarizing microscope in refractive index oil mounts and by scanning electron microscopy. Quenched melt was analysed with the scanning electron microscope and the electron microprobe, both using energy-dispersive analysis.

Melting of phlogopite + quartz + CO₂(50%)H₂O(50%) vap-

our was recognized in 3.4 kbar runs by the presence of glass in isolated globules, crystal-enclosed pockets, thin crystal-boundary linings and as inclusions within enstatite prisms (Fig. 1a,b). The refractive index of the glass was below that of the index oil (1.54) and it appeared distinctly pinkish. The margins of the glass were often devitrified to a yellow substance of moderately high birefringence, undoubtedly mica. The recrystallized quartz contained pink melt inclusions. The appearance of abundant, poikilitic enstatite prisms coincided with the appearance of glass and the decrease or absence of phlogopite (Table 1). A few inclusion-free enstatite needles formed in subsolidus runs by incongruent vapour solution of phlogopite or by dehydration of small amounts of talc component in the synthetic phlogopite⁵. No sanidine was present in these runs. At 6 kbar, glass was not generally present in runs where abundant enstatite formed. Instead, a fine-grained matrix of acicular phlogopite crystals (Fig. 1c) was found, in the same settings as glass in the lower-pressure runs: isolated mats, grain-boundary linings and interstitial patches. This texture is interpreted as quenched melt. Figure 1d shows interstitial quench phlogopite in a partially melted eclogite from a South African kimberlite. The similarity to the experimental quenched liquid is apparent. Large euhedral quartz crystals formed in melted charges and are interpreted as phenocrysts.

A typical glass composition from 3.4 kbar and 785 °C (run No. 254, Table 1) is 66% SiO₂, 20% MgO, 8% Al₂O₃ and 6% K₂O by weight on a restored basis, with probe totals only 60% of the expected counts. This could indicate as much as 40% volatiles; however, some of the deficiency may result from surface irregularities and loss of K₂O, which is probable for very volatile-rich glass (A. T. Anderson, personal communication). A typical composition of glass from 3.4 kbar and 850 °C (run No. 266, Table 1) is 78% SiO₂, 3% MgO, 12% Al₂O₃, and 7% K₂O by weight on a restored basis. This is consistent with the smaller amount of quartz remaining in the 850 °C (run product compared to the 785 °C run product (Table 1). Quenched melt patches from 6 kbar and 770 °C (run No. 253, Table 1) are close to theoretical phlogopite composition.

It may be asked whether the glass observed is actually a vapour precipitate, rather than a quenched liquid. The abrupt

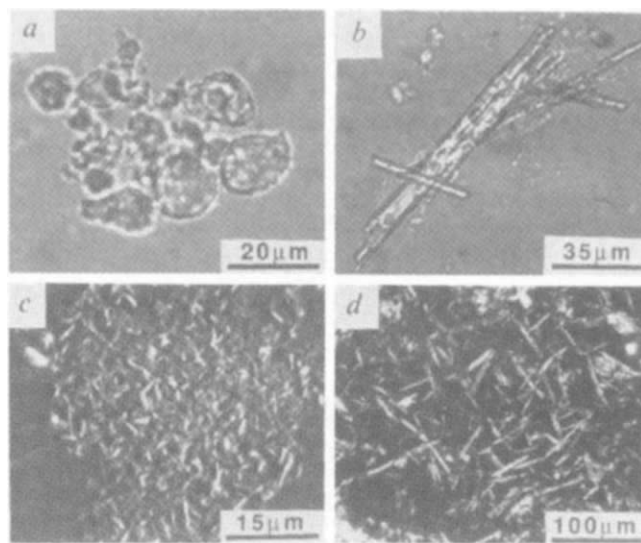


FIG. 1 a, Photomicrograph (ordinary light) of glass globules quenched from 3.4 kbar and 785 °C (run No. 254, Table 1), in equilibrium with quartz-enstatite-vapour ($X_{H_2O} \approx 0.50$); b, Photomicrograph (ordinary light) of enstatite containing glass inclusions (run No. 254, Table 1). c, Scanning electron microscope photograph of fine-grained micaceous quench material from 6.0 kbar and 770 °C (run No. 253, Table 1). d, Polarized photomicrograph (cross-polarized light) of interstitial quench phlogopite in a partially melted eclogite from a South African kimberlite (Barry Dawson No. 1190).

appearance of poikilitic enstatite and glass make a vapour precipitate seem unlikely. Moreover, we reversed phlogopite + quartz melting under excess equimolar $\text{CO}_2\text{-H}_2\text{O}$ at 3.5 kbar in a two-stage run (No. 267, Table 1). The charge was first melted at 785 °C for 283 h and then held at 740 °C for 221 h in the same run (Fig. 2). The quenched charge contained large poikilitic mica crystals grown around granular enstatite cores, and no glass. In another run (No. 275, Table 1) the products from a charge first melted at 6 kbar and 770 °C (No. 253, Table 1), containing enstatite and fine-grained quench material, were held at 3.5 kbar and 783 °C for 162 h. The quenched charge contained abundant glass, enstatite, quartz aggregates and no mica quench material, thus showing the reproducibility of glass formation. At pressures >3.5 kbar, the liquid is apparently too fluid to be quenched to a glass, except in grain boundaries and inclusions.

Metastable melting of phlogopite may be involved, arising perhaps from ultrafine, poorly crystallized starting material. To test this possibility we re-ran, at 3.5 kbar and 785 °C, coarse well-crystallized phlogopite + quartz previously grown at 10 kbar and 770 °C by reversal of hydrous melting⁵. The results were abundant enstatite, pink glass and virtually no remaining phlogopite.

The congruent melting of phlogopite + sanidine + quartz + $\text{CO}_2(50\%)\text{H}_2\text{O}(50\%)$ vapour was also investigated at 6–7 kbar, with quenched-melt textures observed as low as 690 °C. These runs show that congruent melting of biotite-quartz-feldspar can occur under vapour-saturated conditions in the $\text{K}_2\text{O-MgO-Al}_2\text{O}_3\text{-SiO}_2\text{-H}_2\text{O-CO}_2$ (KMASH- CO_2) system at as much as 20 °C lower than in the pure H_2O system (Fig. 2).

Runs 245 and 268 in Table 1 constrain the incongruent melting of phlogopite + quartz + sanidine + vapour between 757 °C and 773 °C at 2.5 kbar. The changes in vapour compositions suggest a buffered composition of mole fraction $X_{\text{H}_2\text{O}} = 0.27$ at this pressure. A calculated vapour composition of $X_{\text{H}_2\text{O}} = 0.23$ is predicted from reversed-equilibrium data on the assemblage phlogopite-quartz-sanidine-enstatite-vapour in the KMASH system⁸ (Fig. 2). These values are close enough to indicate consistency of theory and experiment.

Our results confirm that CO_2 lowers the vapour-saturated solidi of phlogopite-quartz-sanidine assemblages below the pure H_2O solidi. The lowering for the phlogopite + quartz reaction at 3.5 kbar is ~30 °C for a vapour composition of $\text{CO}_2(50\%)\text{H}_2\text{O}(50\%)$ (Fig. 2). Similar behaviour was found at ~4 kbar for pargasite⁹, where the vapour-present solidus is

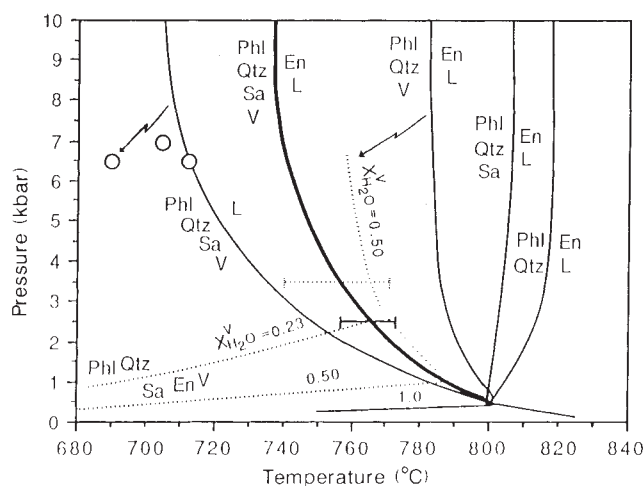


FIG. 2 Univariant equilibria in the KMASH and KMASH- CO_2 systems: Bold curve incongruent melting of phlogopite + quartz + sanidine + vapour from Wendlandt⁴, with bold bracket from present work. This curve represents the trace of the six-phase invariant point phlogopite-quartz-sanidine-enstatite-liquid-vapour in the $\text{H}_2\text{O-CO}_2$ system. Light curves, reactions limiting stability of phlogopite-quartz-sanidine assemblages in pure H_2O system (see ref. 5). Dashed curves, steeply sloping curve is reaction phlogopite + quartz + vapour = enstatite + liquid in $\text{H}_2\text{O-CO}_2$ system, at $X_{\text{H}_2\text{O}}^{\text{V}} = 0.50$, with dashed reversal bracket. The arrow illustrates the displacement relative to the pure H_2O curve. Nearly horizontal curves are calculated positions of reaction phlogopite + quartz = sanidine + enstatite + vapour based on data of Wood⁸, and assuming non-ideal mixing of H_2O and CO_2 in the vapour²¹. Circles, conditions at which phlogopite + quartz + sanidine + vapour melted congruently in the $\text{H}_2\text{O-CO}_2$ system at $X_{\text{H}_2\text{O}}^{\text{V}} = 0.50$. The arrow illustrates the displacement relative to the pure H_2O curve. Phl, phlogopite; Sa, sanidine; En, enstatite; Qtz, quartz; V, vapour; L, liquid.

lowered to 20–30 °C below pure H_2O melting by fluids as rich in CO_2 as 70 mol %. These results imply significant solubility of CO_2 in certain kinds of silicate liquids, mainly alkaline liquids lower in SiO_2 and higher in mafic constituents than granites. The present glass analyses are similar to some syenites¹⁰ and lamprophyres¹¹. Perhaps the magmas from which such rocks crystallized were volatile-rich, with CO_2 nearly equal to H_2O . Experimental partitioning of the two volatiles between alkaline melts and vapour remains an important problem.

TABLE 1 Experimental runs

Run no.	Starting material	wt % vapour	Pressure (kbar)	Temperature (°C)	Time (h)	$X_{\text{H}_2\text{O}}$ (vapour) initial	$X_{\text{H}_2\text{O}}$ (vapour) final	Result
266	Phl + Qtz	50	3.4	850	238	0.50	0.49	En, Gl, (Qtz)
254	Phl + Qtz	50	3.4	785	112	0.50	0.38	En, Qtz, Gl, (Phl)
260	Phl + Qtz	40	3.4	771	283	0.50	0.73	En, Qtz, Gl, (Phl)
277	Phl + Qtz	51	3.5	740	333	0.50	0.50	Phl, Qtz, (En)
267	Phl + Qtz	37	3.5	785 →740	283 →221	0.50	0.49	Phl, Qtz, (En)
255	Phl + Qtz	49	6.0	763	111	0.50	0.53	En, Qtz, Gl, q-Phl, (Phl)
253	Phl + Qtz	58	6.0	770	73	0.50	0.55	En, Qtz, q-Phl
275	Phl + Qtz	48	6.0 →3.5	770 →783	73 →162	0.50	0.51	En, Qtz, Gl
248	Phl + Qtz + Sa	11.7	2.5	757	486	0.00	0.10	En, Qtz, Sa
268	Phl + Qtz + Sa	9.4	2.5	773	429	0.30	0.14	En, Qtz, Sa, Gl
245	Phl + Qtz + Sa	9.7	2.5	757	508	0.25	0.27	En, Qtz, Phl, Sa
241	Phl + Qtz + Sa	10.4	2.5	760	290	0.50	0.40	En, Qtz, Phl, Sa, Gl
162	Phl + Qtz + Sa	50	6.5	714	52	0.50	0.48	Phl, Qtz, Sa, q-Phl, (Gl)
160	Phl + Qtz + Sa	50	6.5	691	48	0.50	0.36	Phl, Qtz, Sa, q-Phl, (Gl)
166	Phl + Qtz + Sa	40	7.0	705	141	0.50	0.46	Qtz, Sa, q-Phl, (Gl)

Phl, phlogopite; Qtz, quartz; Sa, sanidine; En, enstatite; Gl, glass; q-Phl, fine-grained micaceous quench material; (), small to trace amounts present; →, second stage of experiment.

The possibility of generating CO₂-charged alkaline melts in the pressure and temperature ranges of deep-crustal metamorphism opens up several lines of inquiry and potential applications. It is possible that some lamprophyric liquids, possibly precursors to minettes, can be formed in the crust by CO₂ infiltration. Liquids formed by CO₂ melting of biotite in the present study are extremely fluid, as shown by our inability to quench a glass from the higher pressure runs. Such melts might escape upward from the deep crust more easily than granitic magmas; high viscosity of granite melts is now recognized as a fundamental limitation on geochemical differentiation of the crust by partial melting¹². The problem of depletion of large-ion lithophile elements such as K and Rb in some deep-crustal granulite-facies terrains¹³ may be reconsidered in the light of the present results. Rb has small affinity for quartzofeldspathic H₂O-rich liquids relative to biotite or K-feldspar¹⁴, but would probably partition strongly into CO₂-charged melts rich in biotite components, like those of the present study.

Intimate association of syenite and charnockite plutons with granulites in some terrains^{10,15} indicates that they could have formed by anatexis in a deep-crust CO₂-rich environment. The solubility of CO₂ in these melts would be high, probably subequal to that of H₂O, and freezing at shallower levels could generate abundant CO₂-rich fluids, which could create aureoles of granulite facies metamorphism in surrounding rocks, as proposed for the terrain of Wind River, Wyoming¹⁶. The present work sets limits to the possibility of CO₂ streaming through the deep crust, suggested as a fundamental mode of deep-crustal metamorphism¹⁷. The intermediate agency of CO₂ charged silicate liquids in volatile transport through the crust must be considered as an alternative.

These results support those of Wendlandt⁴ in establishing that low-melting CO₂-rich liquids can exist in the temperature-pressure range of deep-crustal processes. A felsic magma containing CO₂ may, under some circumstances, evolve in cooling towards a residual liquid rich in biotite components and CO₂. Freezing of this liquid would release a CO₂-rich vapour. This may explain the CO₂-rich fluid inclusions found in some migmatite leucosomes^{18,19}. A similar application may be made to migmatites and pegmatoids containing unaltered orthopyroxene in some high-grade terrains²⁰. Survival of the orthopyroxene in these rocks is a problem: if the melts were H₂O-rich, as is often supposed, fluids released upon freezing should have altered the orthopyroxene to biotite. The present work suggests that anatectic pegmatoidal liquids may be generated in a CO₂-rich environment; freezing of these liquids might release vapours of reduced H₂O activity which are in equilibrium with orthopyroxene. □

An anthropogenic cause for quaking mire formation in southwestern Ontario

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AMONG the most widely distributed mire types in North America are those maintained by the ground-water table, situated in topographic positions where water accumulates¹. One type is the kettle-hole mire, which develops a buoyant mat over water bodies. Fossil pollen and peat macrofossil evidence indicate that kettle-hole mires in southwestern Ontario in Canada are approximately 150 years old. We hypothesize that deforestation for agriculture by the first European settlers reduced the regulating rates of seasonal runoff which caused the kettle-holes to flood in the spring. Pioneering bog species were placed at a competitive advantage which led to the establishment of floating carpets of vegetation over open water bodies.

Withdrawal of the Late Wisconsin glaciers in the south-central Great Lakes region was characterized by numerous halts and standstills which built a complex series of end moraines. Ice blocks were left, which melted to form numerous undrained kettle-holes on the moraines and associated outwash deposits. Today, mires in the kettle-holes are typified by floating *Sphagnum* mats which encircle or completely cover the water body²⁻⁴.

We have carried out detailed stratigraphic and fossil pollen and peat macrofossil analyses on cores from four kettle-hole mires in southwestern Ontario, where at least 50 mires are concentrated within 100 km² (Figs 1 and 2). The results indicate that human activities initiated hydrological changes, which led to the formation of peatland plant communities, previously assumed to be natural²⁻⁵.

Pollen and peat macrofossil analyses on cores from each of the study sites (Figs 3 and 4) show co-occurrence of major increases in *Sphagnum* spores and macrofossils at about the same horizon as the rise in *Ambrosia* pollen. Pollen of other typical bog species such as *Larix*, *Ericaceae*, *Ilex/Nemopanthus*, *Sarracenia purpurea*, *Drosera*, *Umbelliferae*, *Selaginella selaginoides*, *Menyanthes trifoliata* are present or achieve their highest proportions in this interval also. Agricultural and weedy pollen types, such as *Zea mays*, *Nicotiana tabacum*, *Canabasis/Humulus*, *Rumex*, *Artemisia* and *Plantago* confirm further local land disturbance and forest clearance around the study sites.

Land survey and historical records indicate that settlement in the area of study commenced between AD 1816 and 1830 at an accelerated rate following the depression after the war of 1812 (refs 6 and 7). The settlers initially cleared the land for homestead and farm. The demand for and the price of wheat increased between 1853 and 1854 because of the war with Russia, thereby expanding agricultural activity and forest clearance. Historical records indicate major spring floods and freshets in local rivers starting as early as 1833. Dam building was also important during this period to ensure the year-round operation of mills, much of which ceased during the nineteenth century because streams and small rivers had dried up⁸.

The decline in tree pollen types and increase in *Pinus* and herb pollen, especially *Ambrosia* pollen, are probably the most reliable biostratigraphic indicators of deforestation for agriculture by European settlers in eastern North America. The varved sediment and detailed pollen record from Crawford Lake, a meromictic lake about 50 km east of the study area, indicates that the *Ambrosia* pollen rise began between AD 1846 and 1851, which agrees with historical records for the area^{6,9}.

We hypothesize that clearance of virgin forests for agriculture

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- Kadik, A. A. & Lukanin, O. A. *Geochim. Int.* **10**, 115-129 (1973).
- Eggler, D. H. & Kadik, A. A. *Am. Miner.* **64**, 1036-1048 (1979).
- Holtz, F., Ebadi, A., Barbey, P., Johannes, W. & Pichavant, M. *Terra Cognita* **8**, 66 (1988).
- Wendlandt, R. F. *Am. Miner.* **66**, 1164-1174 (1981).
- Peterson, J. W. & Newton, R. C. *J. Geol.* **97**, 465-485 (1989).
- Puziewicz, J. & Johannes, W. *Contr. Miner. Petrol.* **100**, 156-168 (1988).
- Holloway, J. R. in *Research Techniques for High Pressure and High Temperature* (ed. Ulmer, G. C.) 217-258 (Springer, New York, 1971).
- Wood, B. J. *Prog. exp. Petrol., Wat. environ. Res. Coun.* **6**, 17-19 (1976).
- Holloway, J. R. *Geochim. cosmochim. Acta* **37**, 651-666 (1973).
- Ormaasen, D. E. *Lithos* **10**, 291-310 (1977).
- Rock, N. M. S. in *Alkaline Igneous Rocks* (eds Filton, J. G. & Upton, B. G. J.) 191-226 (spec. Pub. geol. Soc. 30, 1987).
- McKenzie, D. *Earth planet Sci. Lett.* **74**, 81-91 (1985).
- Heier, K. S. *Phil. Trans. R. Soc. Lond. A* **273**, 429-442 (1973).
- Lamb, R. C., Smalley, P. C. & Field, D. J. *metamorphic Geol.* **4**, 143-160 (1986).
- Ouzegane, K., Fourcade, S., Kienast, J.-R. & Javoy, M. *Contr. Miner. Petrol.* **98**, 277-292 (1988).
- Frost, B. R. & Frost, C. D. *Nature* **327**, 503-506 (1987).
- Newton, R. C., Smith, J. V. & Windley, B. F. *Nature* **288**, 45-50 (1980).
- Olsen, S. N. *Contr. Miner. Petrol.* **96**, 104-120 (1987).
- Touret, J. & Dietvorst, P. *J. geol. Soc. Lond.* **140**, 635-649 (1983).
- Waters, D. J. *J. metamorphic Geol.* **6**, 387-404 (1988).
- Kerrick, D. M. & Jacobs, G. K. *Am. J. Sci.* **281**, 735-767 (1981).

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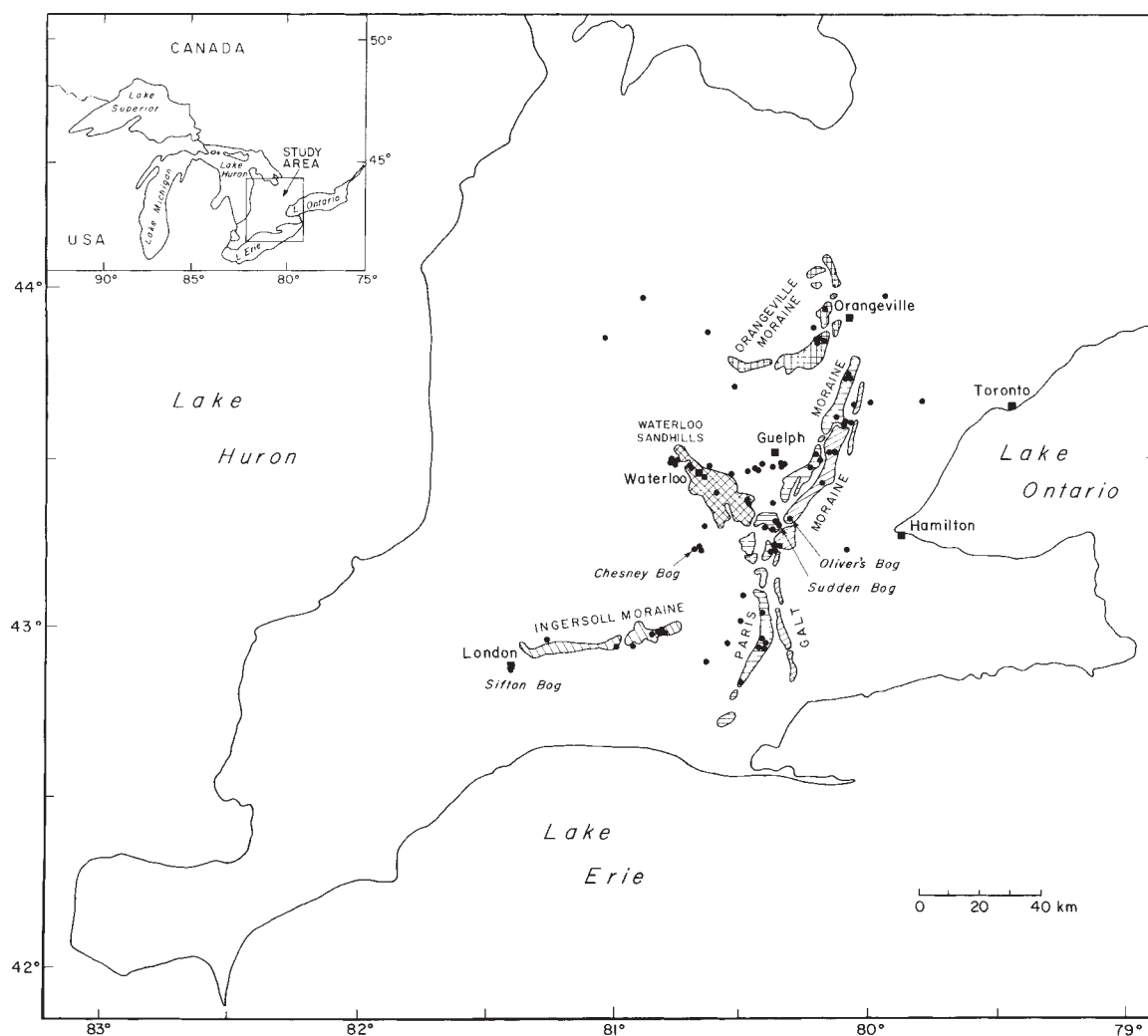
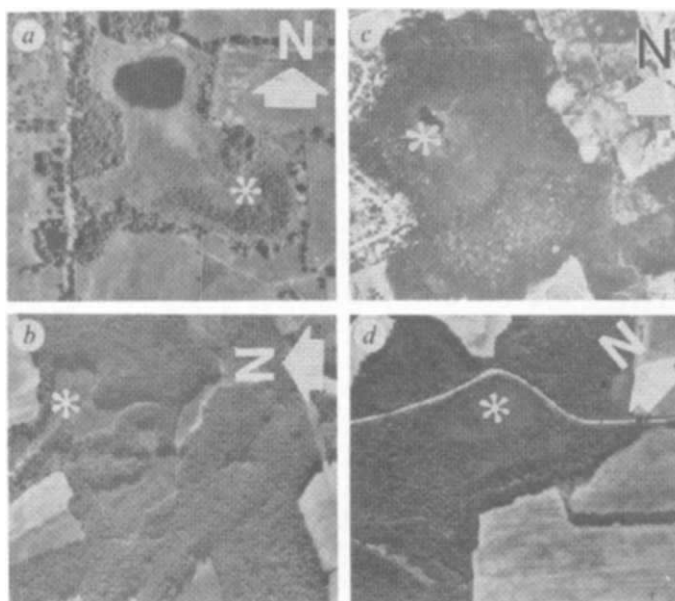


FIG. 1 Location of the four quaking mire study sites in relation to major moraine systems¹⁵ and the distribution of other quaking mires (dots) in southwestern Ontario, Canada.

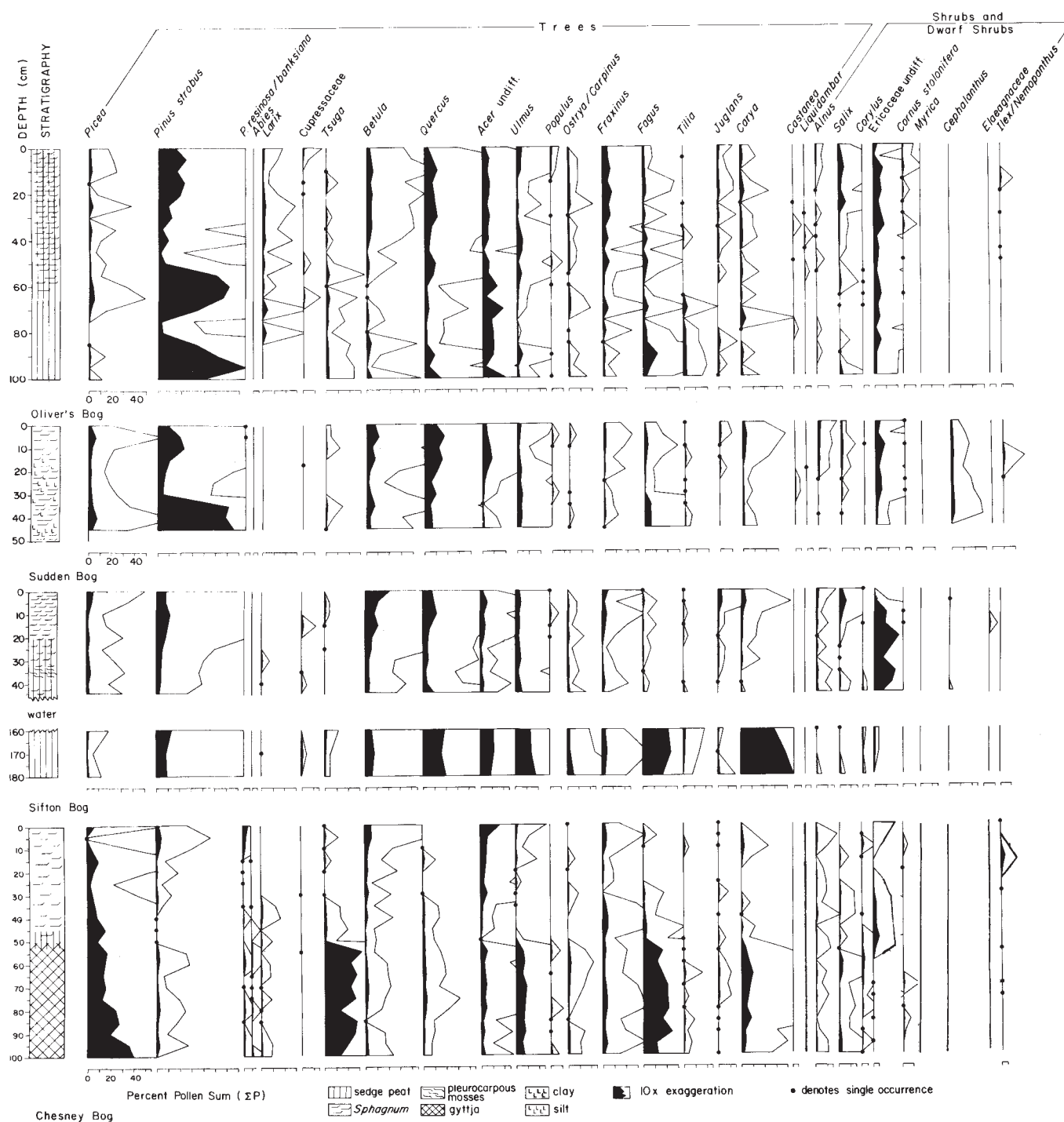
FIG. 2 Aerial photographs of the mire study sites. The asterisks indicate coring sites for the diagrams in Figs 3 and 4. Note the extent of cleared or urbanized land in close proximity of each mire. a, Oliver's Bog; b, Sudden Bog; c, Sifton Bog; d, Chesney Bog. Scale, 1:10,000.



by the first European settlers reduced the regulating effects of forest cover on snow melt during the spring thaw, resulting in increased surface runoff and spring floods in undrained kettle-holes, or a rise in the local groundwater table which was due to diminished loss through evapotranspiration by surface vegetation, or both. Furthermore, elevated water levels could have caused a major vegetation regression in the natural succession of mire communities by eliminating hydrologically sensitive species, or by placing certain species at a competitive disadvantage, so allowing more aggressive bog shrub pioneers such as *Chamaedaphne calyculata*, *Andromeda glaucophylla*, *Potentilla palustris*, *Cephalanthus occidentalis* or *Decodon verticillatus* to flourish within the basin. These plants, in turn, provided a

foundation for the securing of *Sphagnum* mosses, which led to the establishment of floating carpets of vegetation over open water bodies^{1,3,10,11}.

Our interpretation of increased basin water levels supports early ideas that water level change had dramatically affected natural successional patterns of kettle-hole mire plant communities. Theories revolved around changes in water levels resulting from damming by slides, beavers or peat growth, from progressive mat growth sunk by its own weight and size below basin water levels, or from the tearing away from the bottom of continuous mats across the basin, buoyed up as a floating mat by a rise in water levels. Some ecologists proposed that basin water levels were lowered from erosion at an outlet, and



others suggested that both rising and falling water levels affected vegetation patterns differently throughout the basin¹⁰⁻¹³. Although some retrogressive effect on the vegetation was recognized as being due to water level changes, the timing was uncertain. Our studies confirm Waterman's suggestion¹³ that

"... there are indications that it [water level change] may have been due to the activities of the first white settlers in the region, in clearing the forests and in general drainage operations".

We have shown that quaking mires in kettle-holes in south-western Ontario are largely man-made ecosystems of about 150

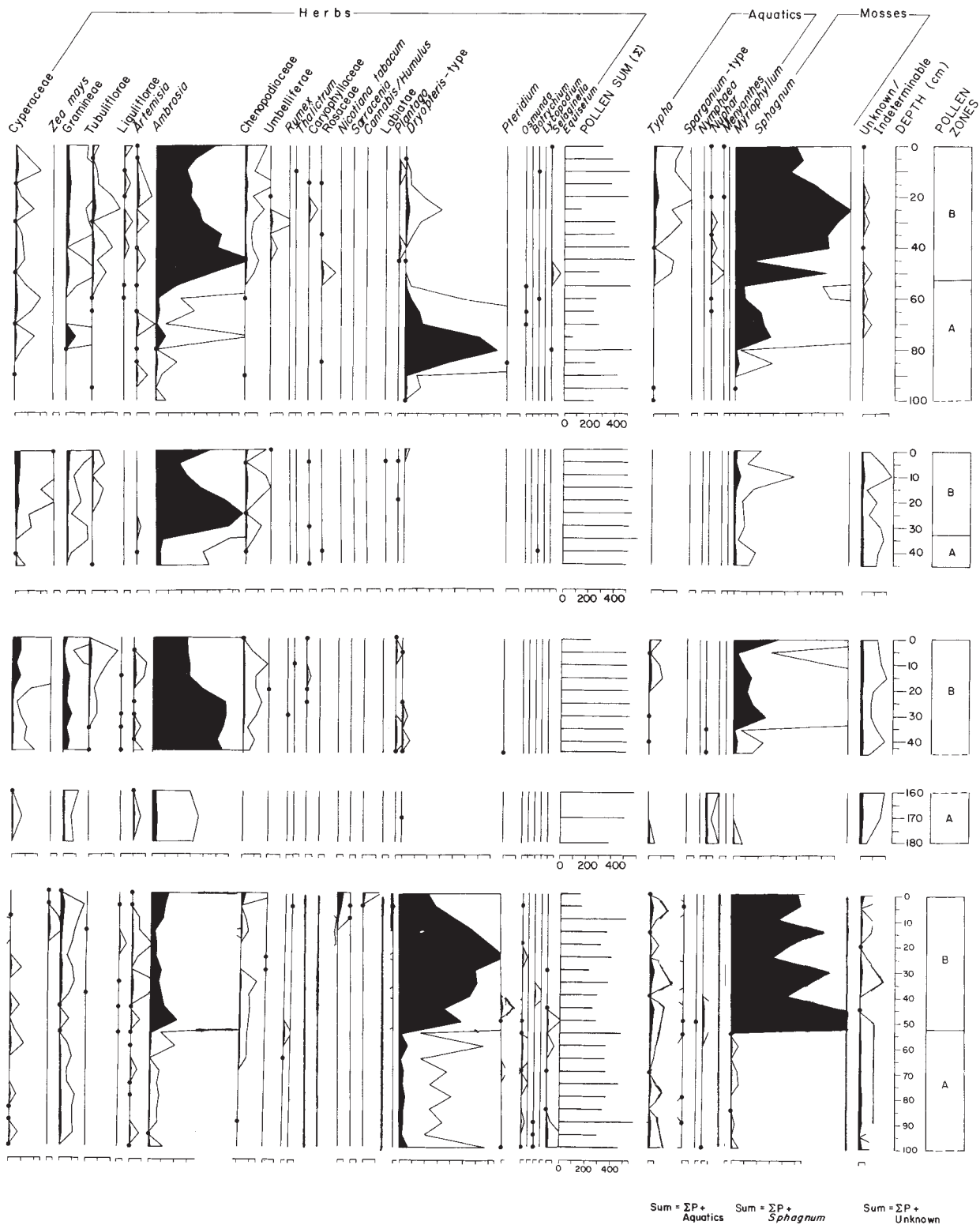


FIG. 3 (Opposite and above) Relative frequency pollen diagrams. Pollen zone A represents the pre-Ambrosia period which contains high proportions of forest tree taxa, especially *Pinus strobus*. Zone B represents the period after land clearance which contains high proportions of weedy and her-

baceous taxa of open fields and forest clearings, and anthropogenic pollen indicators such as *Rumex*, *Plantago*, *Zea mays*, *Nicotiana tabacum*, *Cannabis/Humulus*, and especially *Ambrosia*. Pollen of forest dominants is much reduced in Zone B.

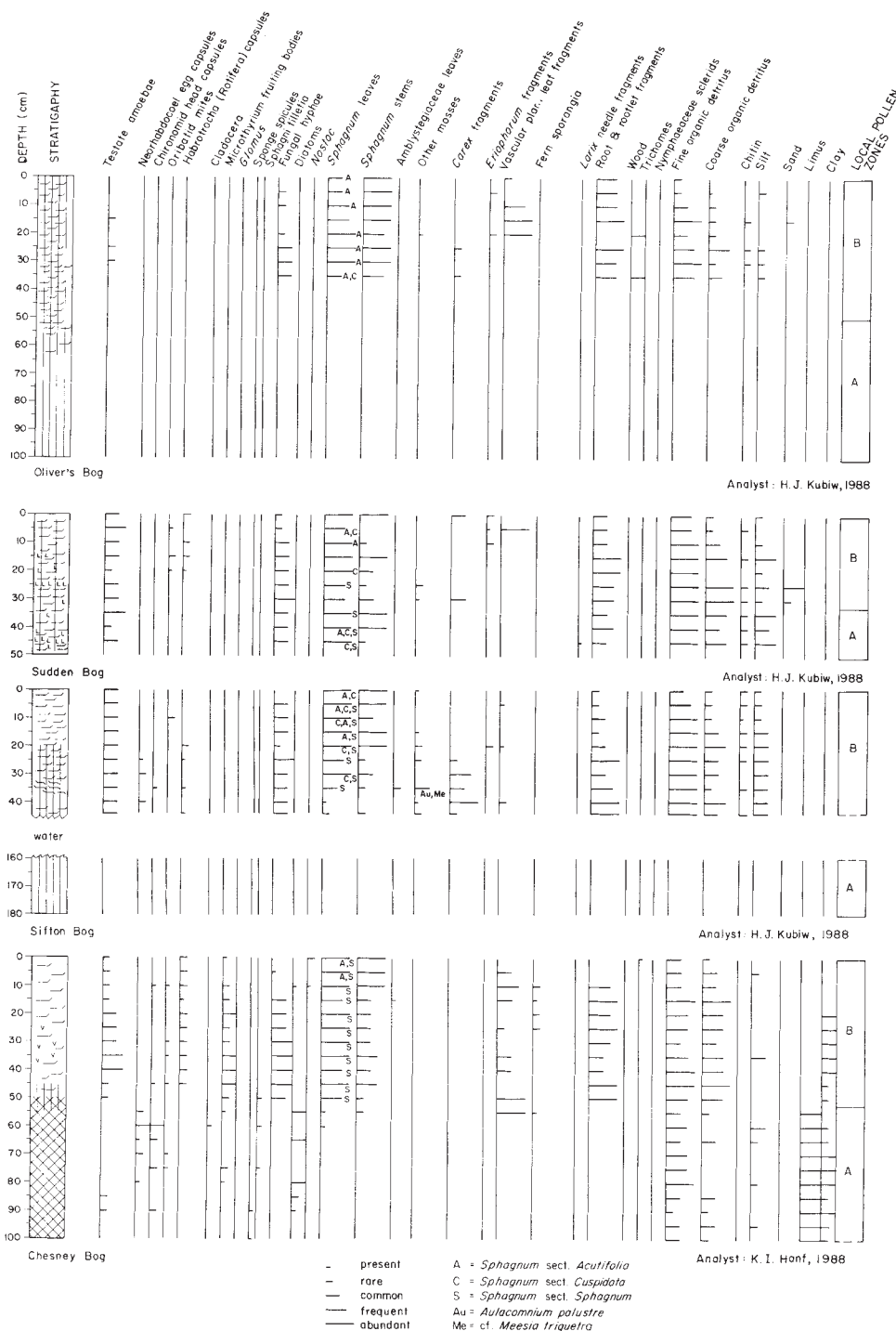


FIG. 4 Peat composition diagrams for the same cores used for pollen analysis in Fig. 3. The occurrence of *Sphagnum* spores is poorly represented in most cores compared with the presence of *Sphagnum* peat components in the cores. A, *Sphagnum* sect. *Acutifolia*; C, *Sphagnum* sect. *Cuspidata*; S, *Sphagnum* sect. *Sphagnum*; Au, *Aulacomnium palustre*; Me, cf. *Meesia triquetra*.

years old. Human land-use activities were important in the establishment of floating carpets of vegetation over open water bodies to form floating mires in Britain¹⁴. The floating mires in Ontario demonstrate that human land-use activities is a widespread feature of floating mire formation. Human activity is one factor in bog development not considered previously in North America. □

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1. Damman, A. W. H. & French, T. W. *US Fish & Wildlife Serv., Biol. Report* **85**(7.16) (1987).
2. Moore, P. D. & Bellamy, D. J. *Peatlands* (Springer, New York, 1974).
3. Crum, H. A. *A Focus on Peatlands and Peat Mosses* (University of Michigan Press, Ann Arbor, 1988).
4. Johnson, C. W. *Bogs of the Northeast* (University Press, New England, Hanover, 1985).

5. Rigg, G. B. *Plant World* **19**, 310–325 (1916).
6. Boyko, M. thesis, Univ. Toronto (1973).
7. Gentilcore, R. L. & Donkin, K. *Can. Cartogr.* **10**, Suppl. 2, 1–116 (1973).
8. Janusas, S. E. *The Cultural Implications of Drainage in the Municipality of Waterloo* (Regional Municipality of Waterloo, 1988).
9. McAndrews, J. H. in *Vegetation History* (eds Huntley, B. & Webb, T.), 673–697 (Kluwer Academic, Dordrecht, 1988).
10. Nichols, G. E. *Bull. Torr. Bot. Club* **42**, 169–217 (1915).
11. Rigg, G. B. *Bot. Rev.* **6**, 666–693 (1940).
12. Dachnowski, A. P. *Plant World* **15**, 25–39 (1912).
13. Waterman, W. G. *Ecology* **7**, 255–272 (1926).
14. French, C. N. & Moore, P. D. *New Phytol.* **102**, 469–482 (1986).
15. Chapman, L. J. & Putnam, D. F. *The Physiography of Southern Ontario* 3rd ed. (Ont. Geol. Surv., Toronto, 1984).

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Motion-deblurring in human vision

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If photographs are taken of moving objects at slow shutter speeds the images of the objects are blurred. In human vision, however, we are not normally conscious of blur from moving objects despite the fact that the temporal response of the photoreceptors is sluggish¹. It has been suggested^{2,3} that there are motion-deblurring mechanisms specifically to aid the visual system in the analysis of the shape of retinally moving targets. Models of motion deblurring have been influenced by the finding that certain very precise spatial pattern discriminations are unaffected by motion. An example is vernier hyperacuity, in which the observer must detect the direction of offset between two lines with abutting ends. With a stationary stimulus, observers can detect a vernier cue of less than 10 arcsec and acuity is unaffected by retinal-image motion of up to 3 deg s⁻¹ (ref. 4). We confirm this finding, but provide evidence against any general deblurring mechanism by showing that another kind of hyperacuity, discrimination of the distance between two parallel lines (spatial interval acuity), is interfered with by motion. This argues against a general deblurring mechanism, such as a neural network 'shifter circuit'³, and we point out that the high level of vernier acuity for moving stimuli is susceptible to an alternative explanation.

We measured thresholds for both vernier and spatial-interval acuity by determining the accuracy of the observer's performance over a range of cue sizes. In the spatial-interval experiment, the observer's task was to decide whether the horizontal spatial separation between two vertical lines was greater or smaller than a standard interval. We used a version⁵ of the task in which the standard separation was the mean of the whole set of stimuli to which the observer was exposed. On each trial, the separation was chosen randomly from a set of predetermined values and the observer had to decide whether it was larger or smaller than the mean of the set; they were informed by an auditory feedback signal whether their response was right or wrong. Such a discrimination is rapidly learned and error thresholds are less than 5% of the standard interval.

The stimuli were presented on a high-resolution oscilloscope screen (Hewlett-Packard 1333A) for an exposure duration of 150 ms. During this brief exposure, the stimuli were either stationary on the screen or moving horizontally in discrete steps of 4-arcmin step⁻¹ (a form of apparent motion difficult to distinguish from a continuous displacement). In the stationary condition the stimulus appeared in the position previously occupied by a fixation point. In the moving case, the stimulus appeared randomly to the left or right of the fixation point and swept across the screen to a position symmetrically on the other side of the fixation point. The amplitude of the trajectory increased in proportion to the target velocity, since exposure duration was kept constant. The 150-ms exposure was too short for the observer to track the object by smooth eye pursuit^{6,7}. To prevent the observer from basing a judgement on the separation between the target lines at the end of their trajectory, a cardboard mask on the screen was positioned so as to make the final spatial position of the leading target invisible. This ensured that the final positions of the two target lines could not be directly compared.

Thresholds were separately determined for a range of stimulus velocities from zero (stationary control) to 6 deg s⁻¹. The technique of threshold measurement by an adaptive method of constant stimuli and probit analysis has been described elsewhere⁷ and is summarized in Fig. 1 legend. In the first experiment, the standard interval was 4.5 arcmin. For comparison, vernier offset thresholds were determined for the same pair of lines, now placed in vertical alignment, over the same velocity

range. The observers were one of us (S.B.) and two others, unaware of the purpose of the experiment. Experiments were conducted under photopic adaptation conditions, and with the luminance of the lines equivalent to a calibration square on the oscilloscope screen of 100 cd m⁻².

The results of the first experiment (Fig. 1a) confirm previous reports that vernier acuity is little affected by target motions up to 6 deg s⁻¹. However, spatial interval thresholds are markedly affected by movement, rising about threefold over the range tested (Fig. 1b). We conclude that insensitivity to image motion is not a general property of hyperacuity, and that the striking ability of observers to perform vernier alignment with moving stimuli does not depend on a general deblurring mechanism.

In considering the effects of motion on perceived shape it is important to distinguish between two issues. First, are there mechanisms to prevent the perception of smear caused by temporal integration of a moving image; and second, is prevention of subjective smear a necessary prerequisite for performance of fine spatial tasks? Burr¹ has demonstrated that perceived motion smear is indeed suppressed in certain circumstances. It is tempting to link this finding to the performance observed with moving vernier-acuity tasks, but the experiments reported here argue against such a connection. If subjective deblurring really prevents the loss of information in the stimulus because of temporal

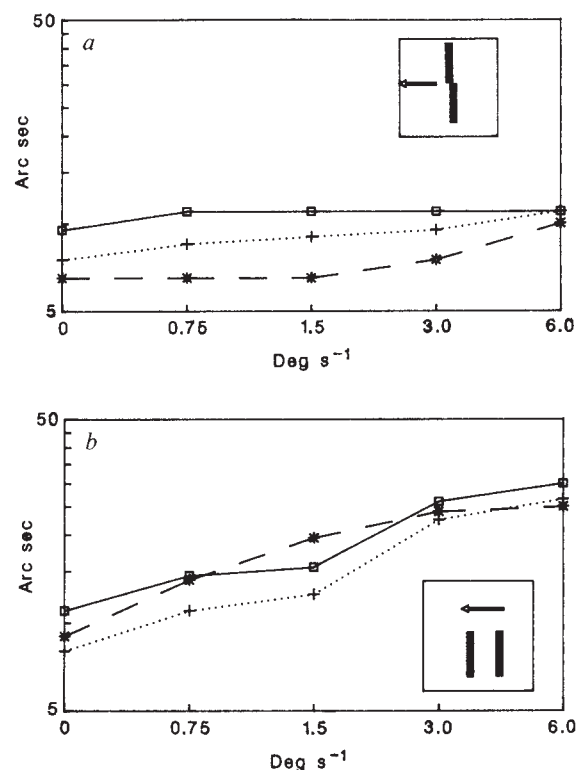


FIG. 1 Thresholds for vernier (a) and (b), spatial-interval acuity tasks (b) as a function of stimulus velocity. The three sets of data points refer to three different observers. In all cases the stimuli are vertical bars moving horizontally. In the vernier-acuity test the observer had to decide the direction of offset between two abutting lines; in the interval-acuity test the task was to compare the length of the gap between the two lines to a 4.5-arcmin-wide standard (b). In the control condition (zero velocity) the stimuli were stationary; in the moving conditions the target swept once across the display screen in a random direction. The threshold is defined as the standard deviation of the best-fitting cumulative gaussian to the psychometric function, which plots the probability of a 'wider' response against the size of the cue. The range from which the cue was randomly chosen was periodically adjusted during each threshold determination to give the greatest information about the shape of the function. Several thresholds were determined under each condition to check for consistency and the final values reported are the r.m.s. values of these measurements.

integration, there should be motion insensitivity to all, not just some, hyperacuity judgements.

Why is vernier acuity so little affected by movement? An important factor may be that the trajectories of the two lines do not overlap on the retina: the instantaneous positions of the two lines could therefore be compared by a mechanism that collects information over the whole extent of the motion-blurred image^{8,9}. Consider, for example, a population of single units with orientationally specific receptive fields stimulated by a horizontally moving vernier target. A vertically oriented receptive field will be stimulated by the two lines of the target with a relative delay and would thus receive less stimulation than a slightly tilted field, which will be stimulated by the two lines simultaneously⁹. Although the pattern of stimulation arising from each of the moving lines is blurred, their peaks are still separately available to signal the inter-bar separation. It is not necessary that features should be discontinuous in their light distribution for the distance between them to be judged accurately, as is clear from studies of spatial-frequency discrimination using sinusoidal gratings¹⁰.

When the two targets follow the same trajectory on the retina and when they are sufficiently close together, movement will blur them to a point where they can no longer be resolved. There is existing evidence that for separations in the region of 4 arcmin, observers base their judgments of spatial interval for static stimuli on the depth of the luminance valley between the two bars^{11,12}. For example, discrimination for stimuli of this size, but not of larger separations, are severely disrupted if the two lines are of opposite luminance contrast¹². The usefulness of the luminance-valley cue will be reduced by image motion, which will blur the two lines into a single-peaked distribution. Our subjective observations agree with this interpretation: even at low velocities the two lines comprising the 4.5-arcmin interval stimulus blurred into a single impression, in which it was difficult to see two lines.

Temporal integration would be expected to demodulate high spatial frequency components having an orientation orthogonal to the direction of motion; spatial frequency components parallel to the direction of motion will be unaffected. The high spatial frequencies in two closely spaced bars will be demodulated by motion, preventing accurate encoding of their spacing. The vernier target, on the other hand, is broad-band both in orientation and frequency, and encoding of the direction of offset does not depend exclusively on high spatial-frequency

components orthogonal to the direction of motion. This explains why the interval discrimination, but not the vernier, is disrupted by motion. A simple prediction is that interval performance would not be disrupted by motion parallel to the bars and in a separate experiment we found this to be the case.

A further prediction is that spatial interval acuity will be less adversely affected by image motion if the standard separation between the bars is increased. Increasing the separation will mean that the two bars are increasingly resolved, despite being spread out by motion blurring. We tested this prediction by using standard intervals of 9, 18 and 36 arcmin. The observers were the two of us and two other naive observers. Our results confirm the prediction (Fig. 2). The more widely separated targets are less susceptible to motion. Indeed, thresholds for the 36-arcmin separation show a degree of motion insensitivity comparable to that for vernier acuity. Analysis of variance of these data confirmed the predicted interaction between stimulus velocity and standard interval size ($F = 3.76$; d.f. = 8,24; $P < 0.0056$).

There have been previous reports of constraints on the ability of the visual system to deal with moving targets^{7,13}. If there is a general deblurring mechanism in human vision, it would maintain resolution of even closely spaced lines. The fact that it does not do so leads us to conclude that the striking insensitivity of vernier acuity to motion depends not on the removal of motion blur, but on the fact that motion blur does not degrade the critical information in the stimulus. □

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1. Burr, D. C. *Nature* **284**, 164-165 (1980).
2. Burr, D. C., Ross, J. & Morrone, M. C. *Proc. R. Soc. B* **227**, 249-265 (1986).
3. Anderson, C. H. & Van Essen, D. C. *Proc. natn. Acad. Sci. U.S.A.* **84**, 6297-6301 (1987).
4. Westheimer, G. & McKee, S. P. *J. opt. Soc. Am.* **A65**, 847-850 (1975).
5. Westheimer, G. & McKee, S. P. *Vis. Res.* **17**, 941-947 (1977).
6. Westheimer, G. H. *Am. med. Ass. Archs Ophthal.* **52**, 932-941 (1954).
7. Morgan, M. J., Watt, R. J. & McKee, S. P. *Vis. Res.* **23**, 541-546 (1983).
8. Morgan, M. J. *Phil. Trans. R. Soc. B* **290**, 117-135 (1980a).
9. Morgan, M. J. & Watt, R. J. *Vis. Res.* **23**, 997-1003 (1983a).
10. Campbell, F., Nachmias, J. & Jukes, J. *J. opt. Soc. Am.* **A60**, 555-559 (1970).
11. Klein, S. A. & Levi, D. M. *J. opt. Soc. Am.* **A2**, 1170-1190 (1985).
12. Levi, D. M. & Westheimer, G. *J. opt. Soc. Am.* **A4**, 1304-1313 (1987).
13. Welch, L. & McKee, S. P. *Vis. Res.* **25**, 1901-1910 (1985).

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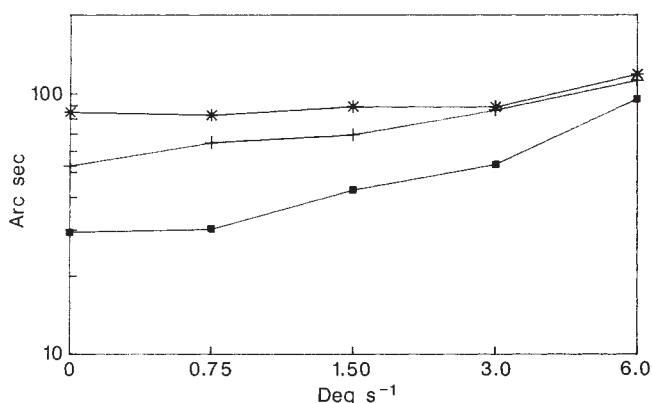


FIG. 2 Thresholds for spatial-interval acuity determined for three standard intervals: 9 arcmin, small square; 18 arcmin, cross; 36 arcmin, star. Results support the prediction that motion would have less of an effect at wider standard intervals. The data points are means for four different observers (M.M., S.B., R.C. and G.H.). The experimental methods were the same as in Fig. 1 except for masking of the ends of the target trajectories. On every trial, each of the target bars was randomly and independently switched off either 0, 4 or 8 arcmin before the end of its trajectory.

The colour centre in the cerebral cortex of man

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ANATOMICAL and physiological studies have shown that there is an area specialized for the processing of colour (area V4) in the prestriate cortex of macaque monkey brain¹. Earlier this century, suggestive clinical evidence for a colour centre in the brain of man^{2,3} was dismissed⁴⁻⁸ because of the association of other visual defects with the defects in colour vision^{4,5,7}. However, since the demonstration of functional specialization in the macaque cortex⁹, the question of a colour centre in man has been reinvestigated,

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based on patients with similar lesions in the visual cortex¹⁰⁻¹². In order to study the colour centre in normal human subjects, we used the technique of positron emission tomography (PET), which measures increases in blood flow resulting from increased activity in the cerebral cortex. A comparison of the results of PET scans of subjects viewing multi-coloured and black-and-white displays has identified a region of normal human cerebral cortex specialized for colour vision.

The increases in blood flow resulting from increased activity in the cerebral cortex can be measured using PET. Our aim was to detect colour-processing areas of the cerebral cortex by presenting normal human subjects with visual stimuli highlighted for colour, and looking for regions of increased activity using PET. Because most stimuli are likely to activate several specialized visual areas, such as those concerned with motion and form, we used an abstract display of coloured squares and rectangles (a 'Mondrian')¹³ to maximize stimulation of areas responsive to colour and minimize the activation of other cortical areas. This display was used on experimental trials (colour trials). For control trials (grey trials) we used a display that was identical, except that the rectangles were of different shades of grey, each grey isoluminant with its coloured counterpart. Isoluminant grey values were determined by converting the relevant colour and its corresponding grey into an alternating moving grating. The luminance of the grey grating was adjusted until the perception of motion ceased¹⁴. We expected that, when compared with the resting (eyes closed) state, activity would be greatly enhanced by the colour stimulus in areas V1 and V2, both of which have compartmentalized groups of wavelength-selective cells¹⁵⁻¹⁷, as well as in the human homologue of V4. The grey display would produce such enhancement only in V1 and V2. As the emission from each grey rectangle included light from all phosphors, the wavelength-selective cells in V1 and V2

were expected to respond to their preferred wavelengths¹⁸.

A PET-scanner (ECAT 931/8/12) (CTI Inc., Knoxville) with an intrinsic spatial resolution of $5.5 \times 5.5 \times 7.0$ mm full-width at half-maximum (FWHM)¹⁹ allowed the simultaneous collection of 15 contiguous transaxial planes, giving a total axial field of view of 10.5 cm. The set of scans was collected so that the lower border of the first transaxial slice was on the orbito-meatal line. Scans were reconstructed using a Hanning filter with a cut-off frequency of 0.5 cycles per pixel, producing a parametric image of cerebral blood flow, with a resolution of $8.5 \times 8.5 \times 7.0$ mm¹⁹. The set of data was then expanded by bilinear interpolation in the axial (z) dimension to produce 43 transaxial slices. This enabled the data to be displayed in a $128 \times 128 \times 43$ voxel matrix, each voxel having similar dimensions in each of the three axes.

Cerebral blood flow was measured using an integral/dynamic technique²⁰. Subjects inhaled trace amounts of $C^{15}O_2$ for 2 min. Dynamic scans, starting 0.5 min before gas administration, were as follows: 1×30 s, 4×5 s, 16×10 s. The scanning sequence therefore spanned brain build-up and wash-out phases of the flow tracer ($H_2^{15}O$ is produced by the transfer of ^{15}O from $C^{15}O_2$ in the lung capillaries^{21,22}). The first 30-s frame was used to correct for residual background activity from previous scans. Throughout scanning, arterial radioactivity was sampled every second by an on-line beta (β^+)-probe. Blood was continuously withdrawn through a small radial-artery cannula at a rate of 5 ml per min. Delay and dispersion of the arterial time-activity curves in the radial artery, cannula and external tubing were measured using a method described previously²³. The corrected arterial input functions were then used to calculate regional cerebral blood flow (rCBF) on a pixel-by-pixel basis, using an integral method applied to the 2-min build-up phase²⁰. Radioactivity distributions were thus transformed into quantitative parametric images of cerebral blood flow.

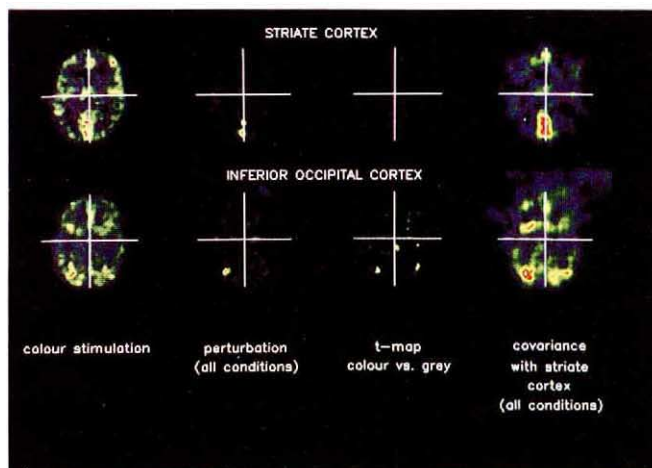


FIG. 1 Parametric and statistical scans at the level of striate and inferior occipital cortices. Colour stimulation: parametric images of rCBF. The striate cortex is 1.2 cm above the AC-PC line and the inferior-occipital cortex 1.7 cm below it. Perturbation: map perturbation (variance) calculated from the pixel-by-pixel grey-value variance across all six scans divided by the difference in grey values between the two rest scans (a reflection of noise). To reduce noise, each scan was smoothed using a low-pass filter of side length 5 pixels (10.25 mm). *t*-Map: *t*-statistic map showing the significance of any difference between colour- and grey-activated scans. To remove variance resulting from changes in global blood flow, the original scans were normalized to a total brain blood flow of $50 \text{ ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$. As the study aimed to locate an area maximally responsive to colour but not grey, a one-tailed *t*-test was used. Covariance map: the covariance of grey values for all six scans with the grey values of a reference pixel located in the striate cortex. White and red represent the greatest activity/significance/covariance, and blue the least.

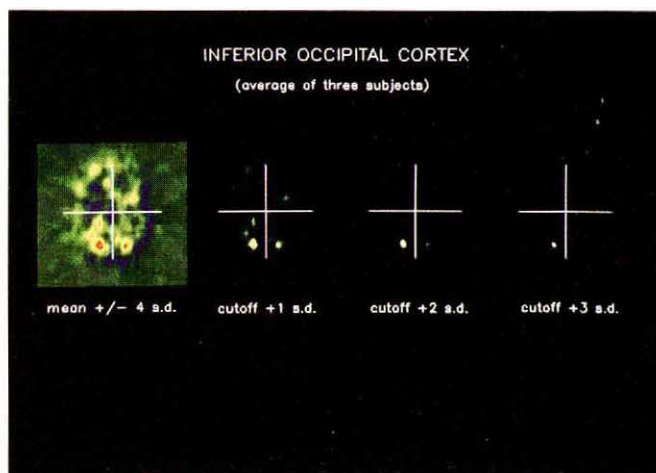


FIG. 2 Standardized difference between colour- and grey-activation scans for inter-subject averaged data. In generating the left-hand scan, the normalized, averaged scans for the stimuli from each subject have been re-sized into a standard-proportional format, permitting pixel-by-pixel inter-subject averaging. The mean grey data were subtracted from the mean colour data after filtering with a 9×9 pixel (18.45×18.45 mm) matrix. This filter was used for inter-subject analysis because of additional variance introduced by normal anatomical variability. The data are in terms of the grey-value difference between conditions (red indicates increased activity during colour stimulation, blue increased activity during grey, and green ~ equivalent activity). Subsequent images have thresholds at 1, 2 and 3 s.d. above the mean, respectively. Plane of section is 6 mm below the AC-PC line.

(a)

Absolute cerebral blood flows (ml 100 ml⁻¹ min⁻¹ to nearest ml)

	Large regions of interest					
	Left			Right		
	Rest	Colour	Grey	Rest	Colour	Grey
Striate	54	64	58	—	—	—
Cortex (midline)	51	60	58	—	—	—
Mean	53	61	58	—	—	—
'Colour area'	53	57	47	51	57	47
	50	60	55	55	60	57
	37	44	36	37	45	39
Mean	47	54	46	48	54	48
Temp.-occ.-parietal pit	42	42	38	44	45	41
	42	52	49	44	51	50
	41	39	37	40	37	36
Mean	42	44	41	42	45	42
Frontal eye fields	44	46	41	44	46	43
	50	50	50	55	57	58
	46	41	41	48	44	43
Mean	47	46	44	49	49	48
Primary motor cortex	47	47	44	49	48	47
	51	52	50	47	48	49
	52	51	49	54	50	48
Mean	50	50	48	50	49	48
Small region of interest						
'Colour area'	50	76	58	45	55	40
	38	55	44	48	56	55
	35	44	36	37	47	43
Mean	41	58	46	44	53	46

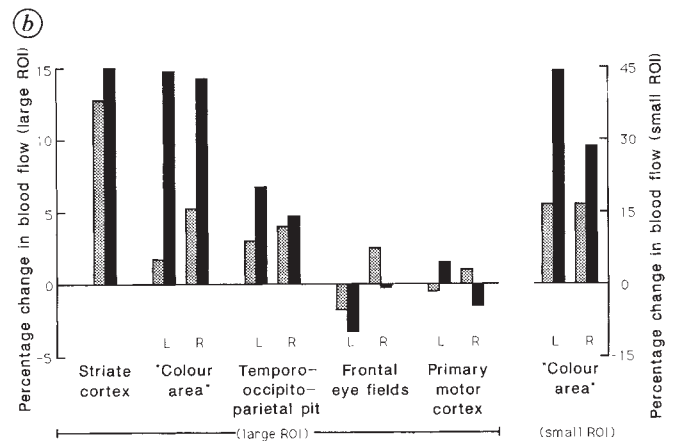


FIG. 3 rCBF and percentage change in rCBF in different regions of the brain. Results are based on absolute cerebral blood flow (a) or on normalized data (b). In b, changes in both colour (black) and grey (stippled) stimulated states are shown as percentage increases over the resting state. The large region of interest (ROI) comprises 180 pixels—1.8 cm in diameter and 0.6 cm in height. Its size is based on the s.d. of the separation of colour areas between different subjects (Table 1). The large ROIs were centred in individual's scans on co-ordinates defined by the region of greatest change observed on the inter-subject averaged, grey-colour difference scan (Fig. 2). Other areas include the striate cortex, frontal eye fields, and primary motor cortex. Also shown are the rCBF and percentage changes in a region around the temporo-occipito-parietal pit, possibly the human homologue of area V5. To assess the maximum change in activity across subjects, a small ROI of 16 pixels (diameter corresponding to the FWHM) was positioned around the areas of maximal colour-induced activity in each subject. L, left; R, right.

Scans were analysed on a computer (SUN 3/60) with image-analysis software (ANALYZE; BRU, Mayo Clinic), which allowed scans to be scaled to standard stereotactic co-ordinates based on the Talairach atlas²⁴ and to be displayed relative to the intercommissural line (AC-PC line). This permitted anatomical localization²⁵ and averaging of data across different subjects.

Three normal male volunteers, of whom two were left-handed, were presented stimuli displayed on a high-resolution colour-television monitor occupying the central 40° of the visual field, corresponding to that represented in V4 (ref. 2). All extraneous light was excluded and other sources of sensory stimulation minimized. To avoid visual fading, subjects moved their eyes to track backwards and forwards along a line (subtending 5°) in the centre of the display. Each subject underwent six scans. Scans 1 and 6 were rest (eyes shut); 2 and 5 were colour trials; and 3 and 4 were grey trials. Each scan lasted 3.5 min, and 15 min separated scans to allow for isotope decay (the half life of ¹⁵O is 2.1 min). Head movement between scans was prevented by the use of head holders of expanded polystyrene.

We analysed our results using statistical methods developed for use with small numbers of subjects. The perturbation studies and covariance maps (Fig. 1) were primarily aimed at finding cerebral areas showing altered activity with visual stimuli and which covaried with activity in the striate cortex. For all subjects, the area of the lingual and fusiform gyri in the inferior occipital region was highlighted by both techniques (Fig. 1).

A pixel-by-pixel map of the *t*-value for the difference between the average of the two colour scans and the average of the two grey scans was constructed for each subject (*t*-map; Fig. 1). The value of the *t*-statistic made the null hypothesis highly unlikely, suggesting that the perturbed areas in the lingual and fusiform gyri were specifically involved in colour processing ($P \leq 0.05$). This was further confirmed by change-distribution analysis of data averaged across individuals (Fig. 2). The precise anatomical locations of the activated areas within these gyri varied

between the individual subjects, reflecting anatomical variability (Table 1).

As expected, there was minimal difference in activity in the striate cortex in response to grey or colour stimulation. We suppose that the area adjoining the striate cortex, area V2, was similarly active in both conditions, but the resolution of the scanner was not fine enough to distinguish the two areas. The only area surrounding the striate cortex consistently showing a significant increase in activity during colour but not during grey stimulation was located in the region of the lingual and fusiform gyri, in agreement with earlier clinical suggestions². We refer to this as the 'colour area', although the determination of colour may not be its only function. We presume this area is the homologue of area V4 in the macaque monkey.

To obtain quantitative data, the activity was sampled from regions of interest defined on the rCBF scans (Fig. 3). In the

TABLE 1 Anatomical location of 'colour areas'

Subject	Side	x (mm)	y (mm)	z (mm)	Distance from mean (mm)
Mean of three	Right	+24	-58	-7	—
	Left	-27	-56	-5	—
Individual brains:					
1	Right	+46	-49	-17	25
	Left	-41	-57	-17	19
2	Right	+10	-49	+5	21
	Left	-24	-56	+3	8
3	Right	+20	-61	-8	5
	Left	-30	-61	-6	6

Distances to nearest mm; reference midpoint of AC-PC line.

striate cortex there was an increase in activity (13–15%) over the resting state for both colour and grey trials (normalized data). An increase of 12–14% was seen in the colour areas during colour but not grey stimulation. There was a 3–5% increase in activity in the region of the temporo-occipito-parietal pit (thought to correspond to area V5 involved in the analysis of visual motion^{26,27}) for both colour and grey trials. Activity in the frontal eye fields and primary motor cortex was little affected by visual stimulation. For normalized data, the average increase in flow in a smaller region of interest centred on the colour area during colour stimulation compared with that during grey was 28% on the left hemisphere and 12% on the right, percentages being those of baseline (rest) flow.

Because of the small increase in flow in V5 seen in our trials, we tested a fourth subject and compared the areas activated by colour stimulation with those activated by a stimulus of black random dots ($1^\circ \times 1^\circ$) moving against a white background at 6° per s, with the direction of motion changing every 15 s. In this subject, an area on the lateral surface of the occipito-temporal boundary, corresponding in position to the human homologue of V5 (ref. 27), showed significantly increased activity during motion presentation. Also, a zone in the colour area showed significant increase during colour but not motion stimulation. This suggests that the increase in activity in the homologue of V5 seen in our initial subjects may have been due to other factors, such as movement of the visual image on the retina generated by eye movements during stimulation.

There were two unexpected results. First, the activity in the colour area of the left hemisphere was greater than that of the right, regardless of whether the subject was left-handed. We are unable to explain this; there is no published suggestion of hemispheric dominance in the analysis of colour. Second, there was no substantial change in activity in the temporal cortex, to which V4 projects in the macaque monkey²⁸. This suggests that the colour area is sufficient for the determination of colour when there is no involvement of memory or experience, an idea requiring further study. In summary, by using modern technology we have been able to demonstrate the existence of a specialized colour centre in the brain of normal man for the first time. □

Ageing and mutation in plants

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PLANTS have many characteristics allowing the accumulation of somatic mutations: lack of a germ line, open systems of growth, flexible meristem organizations and the fact that most somatic mutations are not immediately life-threatening.¹ A consequence of this is that the meristematic initials of a plant accumulate mutations as it ages^{2–4}. We report here that the mutation rates in the long-lived mangrove are 25 times higher than in the annuals barley and buckwheat. An increase in the frequency of mutant initials is, in effect, an increase in the mutation rate per generation, leading to the prediction that long-lived plants will have higher mutation rates per generation than short-lived plants. Because the mutation rate per generation is the primary determinant of inbreeding depression and the dominant and recessive components of genetic load^{5–7}, plant age and/or life span⁸ may be a critical and generally unrecognized aspect of the evolutionary equation.

Estimates of forward mutation frequencies and rates are very rare in long-lived plants⁹. Perhaps the easiest mutant phenotype to detect in higher plants is chlorophyll deficiency or albinism. Based on mutagen studies in barley, the number of nuclear gene loci that can mutate and result in a chlorophyll-deficient phenotype is ~300 (refs 10,11). Thus, this mutant phenotype has a mutation rate that is the sum of the individual mutation rates for each of these loci. Because the genetic organization of the photosynthetic process is ancient and highly conserved, it is likely that a similar number of nuclear gene loci occurs in most angiosperms.

Rhizophora mangle is a diploid ($2n = 36$) woody tree that can reach 25 m in height. The species is unable to reproduce vegetatively, although individual specimens can be widespread because of the support given to lateral branches by 'prop roots' (ref. 12). A peculiar habit of *R. mangle*, as well as other mangrove species, is vivipary, the germination of the seed while still on the tree^{13,14}. The floral biology of *Rhizophora* is characterized by hermaphroditic flowers, anthers dehiscent in the floral bud, and a short functional life of the flower. Primack and Tomlinson¹⁵ hypothesized that mangrove species are self-compatible and more highly inbred than other tropical woody plants. Our results provide strong support for their hypothesis.

Because of self-compatibility and vivipary, *R. mangle* is an ideal organism for mutation studies. The prolonged development of the chlorophyll-containing embryo hypocotyl allows detection of albino embryos while they are attached to the parental tree (Fig. 1). Thus parent and offspring phenotypes and offspring segregation ratios can be measured directly in nature. Table 1 shows segregation data for green and chlorophyll-deficient embryos for 27 different mangrove trees. In 26 trees, the ratio of mutant to green embryos is ~3:1, that is, all of the χ^2 values are nonsignificant.

Summing the data in Table 1 (excluding the presumptive chimaera, J) results in a 3.1:1 ratio of green to white embryos. These segregation data are very compelling evidence that the mangrove populations studied were primarily self-pollinated. If outcrossing occurred to any significant degree, the frequency of mutant embryos per tree should be much less than 25%. Thus a mangrove tree and its attached viviparous embryo can be treated as a controlled breeding experiment in which the parents and offspring phenotype are known.

In Table 2, we present data for an extensive red mangrove population growing in Pigeon Creek, a coastal lagoon on San Salvador Island open to the Atlantic Ocean. This population has a calculated mutation rate for albinism of 7.4×10^{-3} mutations per haploid genome per generation. Red mangrove trees heterozygous for albinism have been found in Florida and Puerto

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1. Zeki, S. M. *Brain Res.* **53**, 422–427 (1973).
2. Verrey, Archs Ophthalmol., Paris **8**, 289–300 (1888).
3. MacKay, C. & Dunlop, J. C. *Scottish med. Surg. J.* **5**, 503–512 (1899).
4. Henschen, S. C. in *Handbuch der Neurologie*, 2 (ed. Lewandowsky, M.) 891–918. (Springer, Berlin, 1910).
5. von Monakow, C. *Gehirnpathologie* Vol. IV (Hölder, Vienna, 1905).
6. Holmes, G. *Proc. R. Soc. B* **132**, 348–361 (1945).
7. Monbrun, A. *Traité d'ophtalmologie* (Société Française d'ophtalmologie, Masson & Cie., Paris, 1939).
8. Duke-Elder, J. A. *System of Ophthalmology* Vol. V (Churchill, London, 1971).
9. Zeki, S. M. *Nature* **274**, 423–428 (1978).
10. Pearlman, A. L., Birch, J. & Meadows, J. C. *Ann. Neurol.* **5**, 253–261 (1979).
11. Damasio, A., Yamada, T., Damasio, H., Corbett, J. & McKee, J. *Neurology* **30**, 1064–1071 (1980).
12. Kölmel, H. W. *Eur. Arch. Psychiatr. Neurol. Sci.* **237**, 237–243 (1988).
13. Land, E. H. *Proc. R. Inst. Gt Br.* **47**, 23–57 (1974).
14. Anstis, S. & Cavanagh, P. in *Colour Vision and Psychophysics* (ed. Mollon, J. D. & Sharpe, L. T.) 155–166 (Academic, London, 1983).
15. Shipp, S. & Zeki, S. *Nature* **315**, 322–325 (1985).
16. DeYoe, E. A. & van Essen, D. C. *Nature* **317**, 58–61 (1985).
17. Hubel, D. H. & Livingstone, M. S. *J. Neurosci.* **7**, 3378–3415 (1987).
18. Zeki, S. *Neuroscience* **9**, 741–765 (1983).
19. Spinks, T. J., Jones, T., Gilardi, M. C. & Heather, J. D. *IEEE Trans. nuc. Med.* **35**, 721–725 (1988).
20. Lammertsma, A. A. et al. *J. Cereb. Blood Flow Metabol.* (suppl.) (in the press).
21. West, J. B. & Dollery, C. T. *J. appl. Physiol.* **17**, 9–13 (1962).
22. Frackowiak, R. S. J., Lenzi, G. L., Jones, T. & Heather, J. D. *J. Comput. Assist. Tomogr.* **4**, 727–736 (1980).
23. Lammertsma, A. A. et al. *J. Cereb. Blood Flow Metabol.* (in the press).
24. Talairach, J. et al. *Atlas d'Anatomie Stéréotaxique du Tégumencéphale* (Masson & Cie, Paris, 1967).
25. Friston, K. J. et al. *J. Cereb. Blood Flow Metabol.* (in the press).
26. Zeki, S. M. *J. Physiol., Lond.* **236**, 549–573 (1974).
27. Mora, B. & Allman, J. M. *Trends Neurosci.* (in the press).
28. Desimone, R., Fleming, J. & Gross, C. G. *Brain Res.* **184**, 41–55 (1980).

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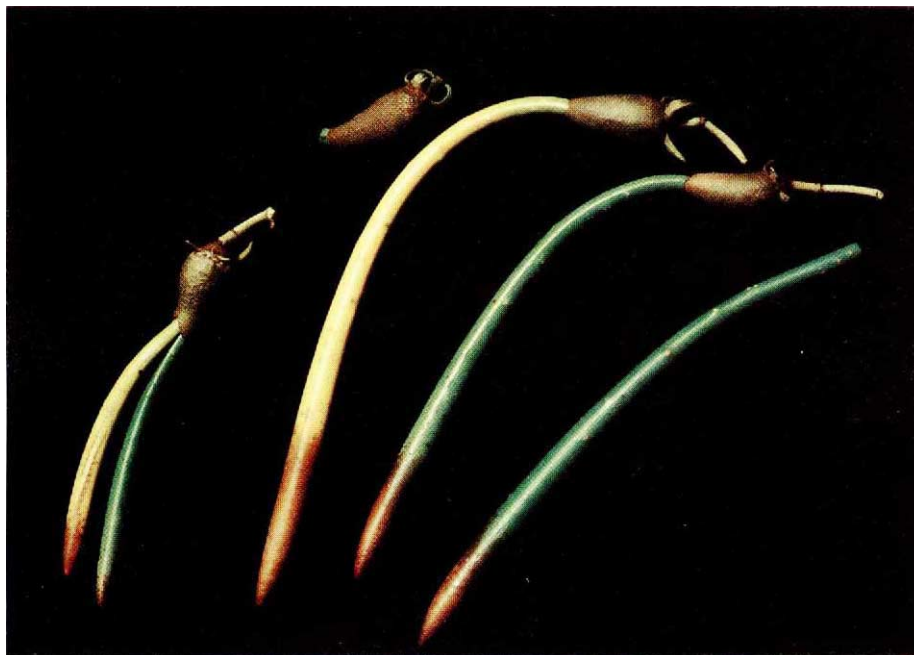


FIG. 1 Albino and green viviparous embryos formed by tree AA in Table 1. Note the rare occurrence of twin seedlings growing from a single ovary. In this instance two ovules were fertilized and two different zygote genotypes and embryo phenotypes were formed, one green and one albino. In *R. mangle*, flowers have two carpels with two ovules each, but normally only one seedling develops per flower. This is due to both prefertilization abortion of some ovules and postfertilization competition²⁶. Viviparous seedling development takes approximately six months and upon maturity a 20–30-cm-long embryo is formed in which the hypocotyl is green and photosynthetic. Vivipary, the prolonged period of embryonic development and selfing allow the easy recognition of sib embryo cohorts and their respective parents.

TABLE 1 Chlorophyll-deficient (white) to green segregation ratios in mangrove

Tree	White	Green	χ^2	P	Tree	White	Green	χ^2	P
A	8	21	0.080	0.5–0.3	O	3	10	0.179	0.7–0.5
C	4	13	0.137	0.3–0.2	Q	1	5	0.667	0.5–0.3
D	3	10	0.179	0.7–0.5	R	5	18	0.275	0.7–0.5
E	2	8	0.400	0.7–0.5	S	7	24	0.204	0.7–0.5
F	3	11	0.314	0.7–0.5	T	1	13	2.952	0.1–0.5
G	4	6	0.933	0.5–0.3	U	3	11	0.286	0.7–0.5
H	18	59	0.169	0.7–0.5	V	7	17	0.167	0.7–0.5
K	5	20	0.520	0.5–0.3	W	1	11	2.333	0.2–0.1
L	41	119	0.025	0.9–0.8	X	1	0	2.333	0.2–0.1
M	3	6	0.259	0.7–0.5	Y	1	5	0.667	0.5–0.3
N	44	123	0.134	0.8–0.7	Z	6	21	0.111	0.7–0.5
AA	2	7	0.259	0.7–0.5	BB	4	15	0.333	0.7–0.5
CC	6	17	0.011	0.9–0.8	J	1	27	7.476	0.01–0.001

χ^2 values and probabilities (P) calculated with Yates' correction and one degree of freedom. Summing all the data (excluding J) results in a 3.1:1 ratio for the 26 heterozygous trees. J is probably a chimaera as only one mutant seedling was present and it was growing at the tip of a small branch. Various chlorophyll-deficient phenotypes were found, paralleling the variety of similar phenotypes seen in barley and other plants. On a single mangrove tree all the chlorophyll-deficient seedlings had identical phenotypes. Sib mutant and wild-type seedlings were grown in the greenhouse; after 10 months all the mutant seedlings exhausted their hypocotyl food reserves and died, whereas the wild-type seedlings continued to grow.

TABLE 2 Mutation frequencies and rates for the Pigeon Creek red mangrove populations in San Salvador

Location	Km*	Seedlings	Approximate number of trees	Heterozygotes	Mutation rate $\mu/2$
South arm, East side	3.7	1,110	111	3	
South arm, West side	3.0	1,011	101.1	1	
Central	1.3	1,174	117.4	4	
North arm, East side	2.8	866	86.6	5	
North arm, West side	1.0	586	58.6	1	
Totals	11.8	4,747	474.7	14	7.4×10^{-3}

Pigeon Creek is a semi-isolated coastal lagoon. Based on pollen data, *Rhizophora* was present in this area at least 5,000 yr BP and the present environment dates to 3,000 yr BP (ref. 25 and J. Teeter, personal communication), presumably sufficient time for the establishment of a mutation-selection equilibrium. Mangroves in this population are rarely over 3 m in height. An average of 10 seedlings per tree was scored. Albinism behaves as a recessive lethal. The mutation-selection equilibrium for the case of complete selfing may be calculated for both individual loci and all loci that contribute to albinism. The derivation of the mutation-selection equilibrium for the case of complete selfing is: $Q' = (P\mu + Q/2)(1 - Q/4)^{-1}$, where μ is the mutation rate per diploid genome, Q is the frequency of heterozygotes in the parental population, P the frequency of wild-type homozygotes, and Q' the heterozygote frequency in the progeny. Because $P = 1 - Q$ and $Q' = Q$ at equilibrium, then $\mu = Q/2(1 - Q/2)(1 - Q)^{-1} \sim Q/2$. The mutation rate per haploid genome is $\mu/2$. Because the generation of homozygotes occurs only within family pedigrees, Q is the sum of the allele frequencies of all loci that may mutate to give rise to albino alleles and μ is the sum of the mutation rates for these loci.

* Shoreline distance sampled

Rico^{16,17}. In a survey of 297 trees at one site along Biscayne Bay, Florida, seven heterozygotes were documented¹⁸. The mutation rate calculated from these data is 5.9×10^{-3} .

When basing estimates of the mutation rate on the frequency of heterozygotes in populations, it is necessary to consider the breeding system, the nature of selection acting upon the homozygotes and heterozygotes, and whether the populations are in a mutation-selection equilibrium. In the mangroves studied, selfing was evidenced by the segregation ratios of heterozygous trees both in San Salvador (Table 1) and Florida¹⁸. The lethality of the albino homozygotes was documented by greenhouse studies and lack of homozygous adults in nature. The neutrality of heterozygotes is evidenced by their normal green phenotypes in nature, although occasional dominant albino mutations have been recovered in other plant species in which heterozygotes are pale green (that is, the yellow soybean locus $Y_{11} y_{11}$ and the sulphur tobacco locus, $Su su$ ¹⁹). Such dominant mutations in mangroves would disadvantage the heterozygotes and result in an underestimate of the true mutation rate. The variety of albino heterozygotes found in populations is evidence against the hypothesis that albino heterozygotes are adaptively superior and represent a simple polymorphism. The length of time Pigeon Creek mangrove populations have existed (>3,000 yr) and the finding of similar mutation rates in San Salvador and Florida favour the mutation-selection equilibrium in these populations.

The mutation rates for chlorophyll deficiency in red mangroves are considerably higher than comparable rates for plants with shorter life spans. The annuals *Hordeum vulgare* (barley) and *Fagopyrum esculentum* (buckwheat) have mutation rates of 3.2×10^{-4} and 3.1×10^{-4} chlorophyll-deficient mutations per nuclear genome per generation, respectively (ref. 20, calculated from Table 4 in ref. 21). Red mangrove mutation rates are ~25 times higher than these values. Although not as well documented as the above estimates, Kirk²² reported that 21 of 81 individuals of a mass-selected variety of *Trifolium pratense* (red clover) were heterozygous for chlorophyll deficiencies. Red clover is a short-lived perennial that ordinarily acts as a biennial²³. The per generation mutation rate calculated from Kirk's data is 5.6×10^{-5} , assuming that mutations at any one of 300 loci may give rise to chlorophyll deficiency.

The higher mutation rates measured per genome per generation in mangroves in contrast to barley, buckwheat and red clover may simply be a consequence of longer mangrove life spans. A generation in barley and buckwheat is one growing season whereas in mangrove it may be 20 or more years. The longer the reproductive life span, the older the meiocytes in terms of the number of cell divisions from zygote and the greater the mutation frequency²⁴. Increases in mutation frequency per sample of gametes in effect increase the calculated mutation rate per generation⁴. □

19. Kirk, J. T. O. & Tilney-Bassett, R. A. E. *The Plastids* (Elsevier, Amsterdam, 1978).
20. Jørgensen, J. H. & Jensen, H. P. *Heredity* **105**, 71–72 (1986).
21. Ohnishi, O. *Jap. J. Genet.* **57**, 623–639 (1982).
22. Kirk, J. T. O. *Scient. Agric.* **5**, 179–186 (1925).
23. Wheeler, W. A. *Forage and Pasture Crops* (Van Nostrand, New York, 1950).
24. Klekowski, E. J. Jr, Kazarinova-Fukshansky, N. & Fukshansky, L. *Am. J. Bot.* **76**, 185–195 (1989).
25. Curran, H. A. (ed.) *Proc. 3rd Symp. Geol. Bahamas* (CCFL Bahamian Field Station, 1986).
26. Juncosa, A. M. *Am. J. Bot.* **69**, 1599–1611 (1982).

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Exclusion of linkage to 5q11–13 in families with schizophrenia and other psychiatric disorders

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RECENTLY a linkage study on five Icelandic and two English pedigrees has provided evidence for a dominant gene for schizophrenia on 5q11–13 (ref. 1). In that study, families with bipolar illness were not included. Using the same probes, two similar but independent investigations on one Swedish pedigree² and on fifteen Scottish families³ excluded linkage to schizophrenia. To evaluate whether the susceptibility gene on 5q11–13 is a common cause of schizophrenia in other populations, we examined five affected North American pedigrees using probes to the *D5S39*, *D5S76* (ref. 4) and dihydrofolate reductase⁵ loci. Two families in the present series had cases of bipolar disorder. We found that linkage can be excluded by multipoint analysis. These results, taken together, suggest that the disease gene on 5q11–13 does not account for most cases of familial schizophrenia.

The five families reported here were selected, from among North American Caucasian families referred to us, on the basis of having the largest number of available relatives with severe psychiatric illness. The diagnostic procedures, and diagnostic definitions using research diagnostic criteria (modified from ref. 6) have been described^{7,8}. Of the eleven schizoaffective individuals diagnosed by these criteria, three would be schizophrenic in *DSMIII-R*.

TABLE 1 Two-point lod scores of linkage of schizophrenia (model 2, 95% penetrance) to 5q11–13 markers

Locus	Family	Recombination fraction				
		0.0	0.05	0.10	0.20	0.30
<i>D5S76</i>	1274	0.17	0.14	0.12	0.07	0.04
	0687	−2.21	−0.40	−0.17	−0.03	0.0
	1257	−3.52	−1.58	−0.97	−0.40	−0.14
	1402	0.06	0.11	0.13	0.12	0.07
	Total	−5.53	−1.73	−0.89	−0.23	−0.03
<i>D5S39</i>	1274	0.06	0.04	0.03	0.02	0.01
	0687	−0.32	−0.24	−0.17	−0.09	−0.04
	1257	−0.37	−0.31	−0.25	−0.15	−0.07
	1402	−1.35	−0.87	−0.60	−0.28	−0.11
	Total	−1.98	−1.37	−1.0	−0.50	−0.20
<i>DHFR</i>	1274	Not informative				
	0687	0.06	0.05	0.04	0.02	0.01
	1257	−2.85	−1.18	−0.72	−0.28	−0.09
	1402	−1.28	−0.69	−0.45	−0.19	−0.07
	Total	−4.09	−1.84	−1.15	−0.48	−0.17

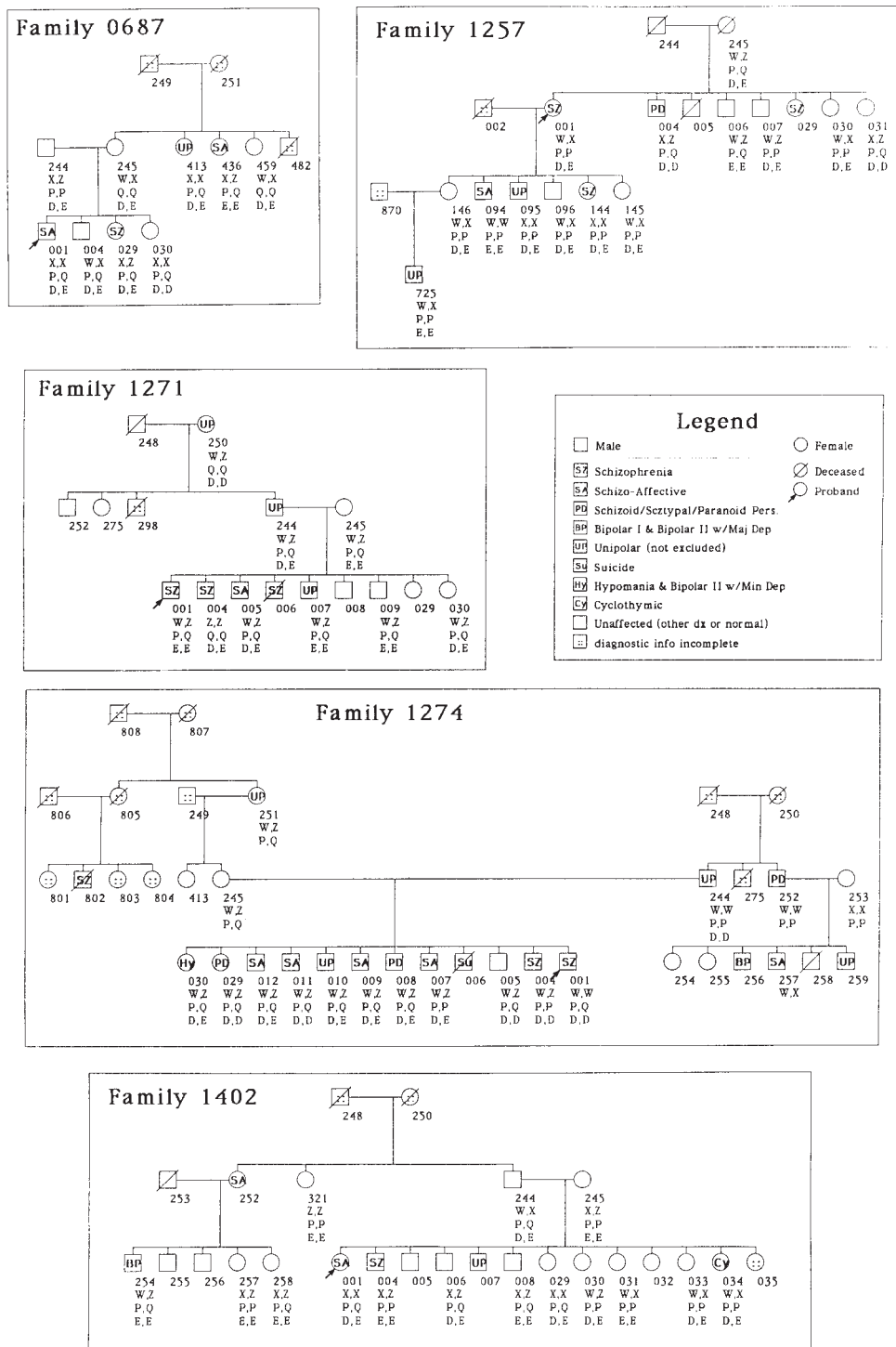
Model 2 includes schizophrenia, schizoaffective disorder and personality disorders (schizotypal, paranoid, schizoid) as affected. Family 1271 is not informative for linkage to any of the markers under model 2.

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1. Klekowski, E. J. Jr in *Proc. XIV Int. Bot. Congr.* (eds Greuter, W. & Zimmer, B.) 137–152 (Koeltz, Königstein/Taunus, 1988).
2. Ledig, R. T. in *Conservation Biology* (ed. Soule, M. E.) 77–104 (Sinauer, Sunderland, Massachusetts, 1986).
3. Klekowski, E. J. Jr, Mohr, H. & Kazarinova-Fukshansky, N. in *Genetics, Development, and Evolution* (eds Gustafsson, J. P., Stebbins, G. L. & Ayala, F. J.) 79–113 (Plenum, New York, 1986).
4. Klekowski, E. J. Jr *Mutation, Developmental Selection and Plant Evolution* (Columbia University Press, New York, 1988).
5. Muller, H. J. *Am. J. hum. Genet.* **2**, 111–176 (1950).
6. Klekowski, E. J. Jr *Heredity* **61**, 247–253 (1988).
7. Charlesworth, D. *Nature* **338**, 21–22 (1989).
8. Wiens, D. et al. *Nature* **338**, 65–67 (1989).
9. Sutherland, W. J. & Watkinson, A. R. *Nature* **320**, 305 (1986).
10. Gustafsson, A. *Radiobiology Symp. Liege Proc.* 1954 282–284 (1955).
11. von Wettstein, E. et al. in *Autonomy and Biogenesis of Mitochondria and Chloroplasts* (eds Boardman, N. K. et al.) 205–223 (North-Holland, Amsterdam, 1971).
12. Gill, A. M. & Tomlinson, P. B. *Biotropica* **9**, 145–155 (1977).
13. Sussex, I. *Am. J. Bot.* **62**, 948–953 (1975).
14. Tomlinson, P. B. *The Botany of Mangroves* (Cambridge University Press, Cambridge, 1986).
15. Primack, R. B. & Tomlinson, P. B. *Biotropica* **12**, 229–231 (1980).
16. Teas, H. J. in *Biosaline Research* (ed. San Pietro, A.) 369–381 (Plenum, New York, 1982).
17. Handler, S. H. & Teas, H. J. *Tasks Veget. Sci.* **8**, 117–121 (1983).
18. Teas, H. J. & Handler, S. H. *Proc. Int. Symp. mar. Biogeogr. Evol. South. Hemis.* **2**, 357–361 (1979).

FIG. 1 Genotypes of *D5S76*, *D5S39* and *DHFR* in pedigrees with schizophrenia. Abridged pedigree structures for families 687, 1257, 1402, 1271 and 1274. The *D* and *E* alleles are the 1.75 and 0.86-kb fragments of *DHFR* respectively. The 6.3 and 5.2-kb fragments are the *P* and *Q* alleles of p105-153Ra (*D5S39* locus) (ref. 4) respectively. The probe p105-599 Ha (*D5S76* locus) (ref. 4) detects 17, 14 and 10-kb alleles which are designated *W*, *X*, *Z*, respectively.

METHODS. Genomic DNA preparation from lymphoblastoid cell lines of pedigree members was as described¹⁷. The digested DNAs were electrophoresed on 0.8% agarose gel in 1×TBE (0.89 M Tris-borate, 0.089 M boric acid, 0.002 M EDTA) and transferred to a nylon membrane (ICN Biotrans or Schleicher and Schuell Nytran) by Southern blotting¹⁸. All DNAs were further typed using lambda MS8, a minisatellite probe provided by Dr A. Jeffries¹⁹. Plasmid cultures of p105-599 Ha and P105-153 Ra (ref. 4) were provided by Dr J. Wasmuth and a 315-bp subclone of the *DHFR* intron by Dr T. Shimada. The clones were propagated according to standard methods²⁰ and the whole clone or released insert was labelled with [α -³²P] dCTP (3,000 Ci mmol⁻¹) to a specific activity of $\sim 2 \times 10^9$ c.p.m. μ g⁻¹ DNA by the random primer method²¹. Hybridization and washing of blots and autoradiography were as described¹⁷. The diagnosis is shown according to the key and the pedigrees were drawn using a program as previously described²².



All diagnostic decisions were made with no knowledge of marker genotypes. In the families studied here, a total of 26 individuals were diagnosed as having either schizophrenia, schizoaffective illness or schizophrenia spectrum disorder (Fig. 1). Additionally, 15 individuals had an affective diagnosis, an observation consistent with the epidemiological data of Gershon *et al.*⁸.

The segregation of the polymorphisms at the *D5S76*, *D5S39* and dihydrofolate reductase (*DHFR*) loci on 5q11-13 was determined in 65 individuals. We have identified two *DHFR* allelic fragments of 1.75 and 0.86 kilobases (kb) using *RsaI* and a 315 base-pair (bp) intron fragment located between exons 1 and 2 as probe (unpublished observations). This pattern is distinct from that observed using a different probe⁹. The intron fragment

specifically maps to 5q11-13 (T. Shimada, personal communication). Recently, Kaufmann *et al.* mapped *D5S39*, *D5S76* and *DHFR* within the trisomic region that has been shown to be associated with schizophrenia in two members of a Chinese family^{10,11}. The genotypes obtained in the present study are shown in Fig. 1.

For two-point and multipoint linkage analyses we used the program LINKAGE, version 4.7 (ref. 12). Individuals were classified as 'affected' for analysis in three different ways. Model 1 included as ill only those individuals with schizophrenia or schizoaffective disorder. Model 2 included these individuals and those with schizotypal, paranoid or schizoid personality disorder, defined as 'schizophrenia spectrum' by Sherrington *et al.*¹. The affected category in model 3 included those in models 1

and 2 plus individuals with unipolar or bipolar affective disorder. Schizophrenia (or other included diagnoses) was assumed to be caused by a dominant gene with a frequency of 0.015 and a maximum penetrance of 95%. Age correction was also applied.

The lod scores by family obtained under model 2 with high penetrance (95%) for loci *D5S76*, *D5S39* and *DHFR* are presented in Table 1. Family 1271 is not informative for any locus under model 2. It is clear that there is no linkage at any of these three loci¹³.

The results from multipoint analysis across the reported linkage region are presented in Fig. 2. The map locations for the markers (sex average) were taken from Weiffenbach *et al.*¹⁴. Under the high-penetrance model for the narrowest disease classification (model 1), linkage can only be excluded (by lod score < -2) at about 5 cM from each marker. If the definition of illness is widened (models 2 and 3), however, the whole region can be excluded, that is, from 10 cM proximal to *D5S76*, up to the *DHFR* locus.

If we reduce the assumed maximum penetrance to 60%, the analysis preserves the information from ill individuals but greatly reduces the linkage information from healthy individuals. In this case, multipoint analysis excludes linkage at each marker but the lod scores are not sufficiently negative to rule out linkage in the region between the loci (analysis not shown).

The exclusion of linkage in the pedigree series presented here concurs with previous reports^{2,3} and apparently differs from the findings of Sherrington *et al.*¹, which revealed that a single dominant gene on 5q11-13 is segregating with schizophrenia. Sherrington *et al.* used a different clinical assessment system in that they excluded any pedigree containing a bipolar case. One could hypothesize that this clinical distinction successfully distinguishes the linked from the non-linked families. However, the lod scores obtained when including the families with bipolar disorder (1274 and 1402), are not more negative than those of pedigrees 687, 1271 and 1257. In the multipoint analysis, the linkage exclusion does not depend on these families (1274 and 1402; data not shown).

Taking all the findings into account, we can tentatively consider schizophrenia as aetiologically heterogeneous, although other interpretations are possible. Genetic heterogeneity in affective disorders¹⁵⁻¹⁷ has also been proposed to account for

disparities in linkage results. The resolution of the varying linkage results may have to await study of a large series of families to resolve even substantial heterogeneity, or a demonstration of a clinical or biological marker that is uniquely associated with 5q11-13 schizophrenia. □

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- Sherrington, R. *et al.* *Nature* **336**, 164-167 (1988).
- Kennedy, J. *et al.* *Nature* **336**, 167-170 (1988).
- St. Clair, D. *et al.* *Nature* **339**, 305-309 (1989).
- Leppert, M. *et al.* *Science* **238**, 1411-1413 (1988).
- Anagnou, N. *et al.* *Proc. nat. Acad. Sci. U.S.A.* **81**, 5170-5174 (1984).
- Spitzer, R., Endicott, J. & Robins, E. *Research Criteria for a Selected Group of Functional Disorders* 3rd edn (New York State Psychiatric Institute, New York, 1978).
- DeLisi, L. *et al.* *Archs gen. Psychiat.* **44**, 891-897 (1987).
- Gershon, E. *et al.* *Archs gen. Psychiat.* **45**, 328-336 (1988).
- Feder, J. *et al.* *Nucleic Acids Res.* **15**, 5906 (1987).
- Kaufmann, C. A. *et al.* in *Schizophrenia Res.* **2**, 42a (1989).
- Bassett, A. *et al.* *Lancet* **i**, 799-800 (1988).
- Lathrop, G. *et al.* *Am. J. hum. Genet.* **37**, 482-498 (1985).
- Morton, N. *Am. J. hum. Genet.* **7**, 277-318 (1955).
- Weiffenbach, B. *et al.* *Am. J. hum. Genet. (Suppl)* **43**, A162 (1988).
- Egeland, J. *et al.* *Nature* **325**, 783-787 (1987).
- Hodgkinson, S. *et al.* *Nature* **325**, 805-806 (1987).
- Detera-Wadleigh, S. *et al.* *Nature* **325**, 806-808 (1987).
- Southern E. *J. molec. Biol.* **98**, 503-517 (1975).
- Wong, Z. *et al.* *Am. J. hum. Genet.* **51**, 269-288 (1987).
- Maniatis, T., Fritsch, E. & Sambrook, J. *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
- Feinberg, A. & Vogelstein, B. *Analyt. Biochem.* **132**, 6-13 (1983).
- Mamelka, P., Dyke, B. & McCluer, J. in *Pedigree Draw for the Apple Macintosh, Version 3* (Southwest Foundation for Biomedical Research, Texas, 1987).

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Genetic analysis of immortalizing functions of Epstein-Barr virus in human B lymphocytes

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EPSTEIN-BARR virus (EBV), a herpes virus, infects human B lymphocytes *in vitro* and efficiently immortalizes them¹. About 10 of the approximately 100 genes of EBV are expressed in recently immortalized B cells and although there is circumstantial evidence that at least three of these may contribute to the process of immortalization, there is no direct evidence that any particular gene is required². We have developed a genetic analysis of EBV that uses a transformation-defective strain of the virus as a helper virus in conjunction with DNA that contains all of the viral *cis*-acting elements required for replication, cleavage and packaging during the lytic phase of the viral life cycle. This DNA can include viral genes required for immortalization that complement the transformation-defective virus strain. The DNA can be amplified and packaged by the products of the helper virus and the packaged DNA is infectious. We have analysed two viral genes expressed in immortalized cells and find that the gene encoding EBV nuclear antigen-2 is required for immortalization, whereas the gene for the EBV nuclear antigen leader protein is not.

The origin of replication of EBV used during its lytic cycle has been identified as *oriLyt*³. The signals required for cleavage and packaging of lytically replicated DNA have not yet been identified. For several herpesviruses the signals for cleavage and packaging of replicated DNA have been located near the termini of their virion DNA^{4,5}. To test whether the cleavage and packaging signals of EBV are similarly located, we cloned a 3 kilobase (kb) fragment containing the fused termini from a circular molecule of EBV DNA into a plasmid containing *oriLyt*. These termini are composed of variable numbers of direct repeats called 'terminal repeats' (TR). The parental plasmid

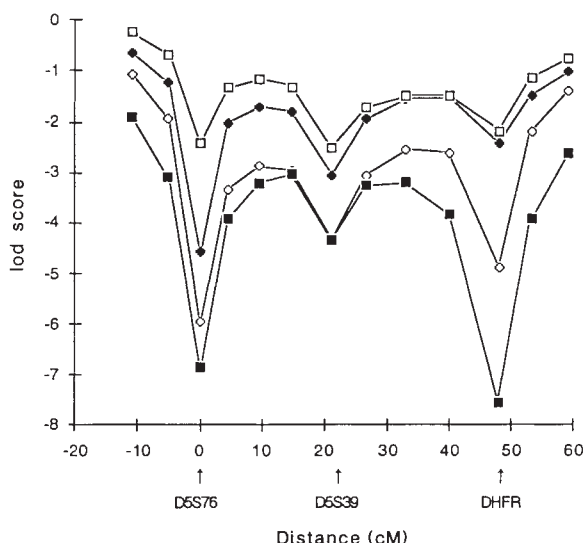


FIG. 2 Lod scores comparing the possible location of a schizophrenia locus relative to three fixed marker loci on 5q, based on genotypes from Fig. 1. Location scores were computed using the LINKMAP program¹² under an autosomal dominant mode of inheritance. ♦, Model 1, schizophrenia and schizoaffective, $f=95\%$; ◇, Model 2, also including schizotypal, paranoid and schizoid, $f=95\%$; ■, Model 3; bipolar and unipolar; (+model 2), $f=95\%$; □, Model 2, $f=60\%$.

p135(TR^-) and the derivative p554(TR^+) carrying the fused termini were separately introduced into a cell line latently infected with EBV, and the lytic phase of the viral life cycle was induced in these cells by exposing them to 12-O-tetradecanoylphorbol-13-acetate (TPA)⁶. After four days the cell culture supernatant was collected and probed for encapsidated DNA and the resulting signal was compared with signals arising from the plasmid-derived DNA within the cells (Fig. 1). Only the plasmid p554(TR^+), which contains the terminal repeat region of EBV, was encapsidated into virions in such a way as for its DNA to be detectable in the supernatant of the induced cells.

To determine whether the packaged DNA derived from transfected plasmids is infectious, we asked whether it could rescue immortalization by an EBV strain, termed P3HR1, which by itself cannot immortalize primary human B cells. P3HR1 lacks the last two exons of the *EBNA-LP* (EBV nuclear antigen-leader protein) gene and all of the *EBNA-2* (EBV nuclear antigen-2) gene⁷. Both of these genes are expressed in cells recently infected by immortalizing strains of EBV. Because both genes are conspicuously absent in this non-immortalizing strain of EBV, both have been considered to be important for immortalization⁸. The plasmid p554(TR^+), packaged as shown in Fig. 1, contains all of the *EBNA-LP* and *EBNA-2* DNA sequences deleted in the P3HR1 strain of EBV (Fig. 2a). The plasmid p554(TR^+) was introduced into the cell line that contains the P3HR1 strain of

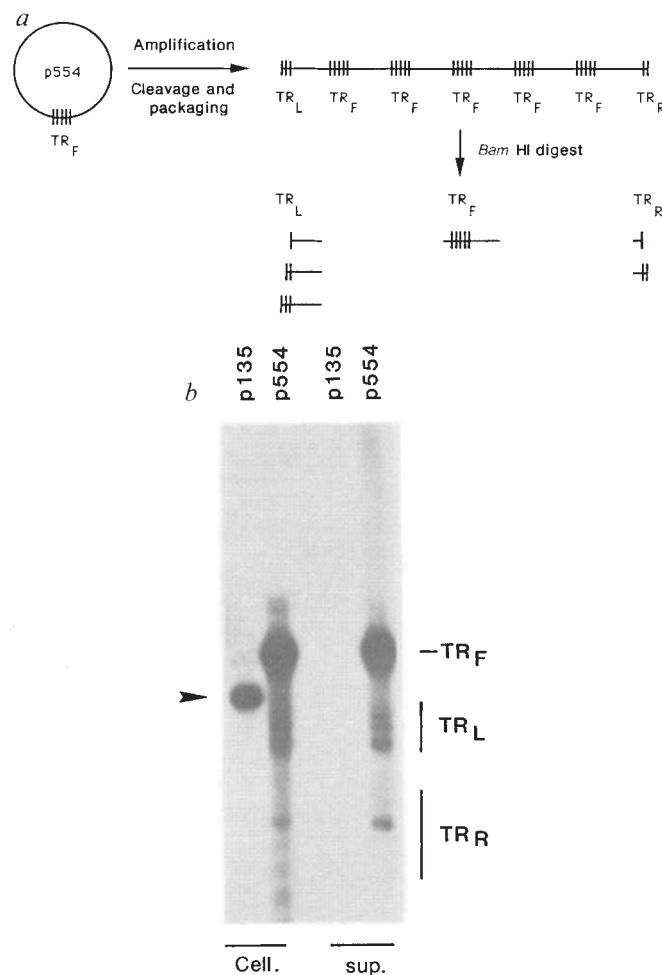
EBV (the cell line is also termed P3HR1) and the lytic phase of the viral life cycle induced by co-transfection with the viral transactivating gene *BZLF-1* (refs 3, 9). The virus stock released from these induced cells consists of the endogenous P3HR1 virus, encapsidated concatemers of p554(TR^+) and possible recombinants between them. This virus stock, derived from P3HR1 plus p554(TR^+), immortalized infected B lymphocytes, whereas a virus stock derived from P3HR1 alone did not (Table 1). Human B lymphocytes showed a dose-dependent immortalization with the virus derived from P3HR1 + p554(TR^+), see (Table 1).

The plasmid p554 (Fig. 2a) contains the plasmid origin of replication, *oriP*, of EBV (ref. 10). A concatemer of p554 could therefore replicate as a plasmid after circularization in cells if the *EBNA-1* gene of EBV, which is required for plasmid replication¹¹, were provided in *trans*. The P3HR1 strain of EBV encodes *EBNA-1* so that both the P3HR1 and concatemeric p554 DNA could be maintained together as plasmids in cells.

We analysed, by Southern blotting¹², DNA from clones of cells immortalized by the P3HR1 + p554 virus stock to identify the composition of the P3HR1- and p554-derived DNA. Clones 1 and 2 of the seven clones analysed contained both the P3HR1 viral DNA and the p554-derived DNA (Fig. 2). Five of the seven clones analysed contained only viral DNA (for example, clones 3 and 4 of Fig. 2) in which the deletion in P3HR1 had been healed, presumably as a result of a double recombinational

FIG. 1 Detection of intracellular and encapsidated extracellular plasmid-derived DNA. *a*, Representation of the concatemeric structure of p554 formed by its amplification, cleavage and packaging into virions. The plasmid p554 is ~29 kb and a 6-mer of it would be 174 kb, which is close to the genomic size of 172 kb. TR_F represents the fused terminal repeats in p554; they are composed of multiple copies of 500 base pair (bp) terminal repeats found at both ends of EBV genomic DNA². The cleavages that accompany packaging of EBV DNA yield variable numbers of terminal repeats at the left end (TR_L) and at the right end (TR_R) of encapsidated molecules. Digestion of a population of linear 6-mers of p554 with *Bam*HI endonuclease yields fragments containing TR_L of variable lengths, one fragment containing TR_F and other fragments containing TR_R of variable lengths. *b*, Replicated plasmid-derived DNA was detected by Southern blot analysis¹² in isolated total intracellular DNA or in purified extracellular, packaged DNA after endonuclease digestion. The two plasmid DNAs are identical in structure, except that p554 contains the terminal repeat region of EBV (its structure is shown in Fig. 2a). The two lanes marked *Cell*, are derived from intracellular DNA, and the two marked *Sup.* are derived from extracellular DNA found in the cell culture supernatant. TR_F identifies signals derived from the fused terminal repeats, TR_L identifies signals from the left terminal repeats, and TR_R identifies signals from the right terminal repeats of extracellular and intracellular p554. The encapsidated 6-mer of p554 should contain five copies of the fused terminal repeats and one copy each of the left and right terminal repeats. Therefore, the intensities of the signals are consistent with the proposed composition of an encapsidated derivative of p554. The arrow identifies the intracellular signal resulting from the cleaved fragment of p135 that is absent in the extracellular DNA preparation. This fragment in p135 corresponds to TR_F , but it lacks 3 kb of DNA containing the fused terminal repeats present in p554.

METHODS. The plasmids p135 and p554 were introduced into the GG68 clone 5 of P3HR1 cells¹⁸ by electroporation¹⁹; 10 μ g each plasmid DNA was introduced into 10^7 cells. Pools of cells carrying this DNA were selected by propagation in 1,500 μ g ml⁻¹ of G418. As p135 and p554 carry the plasmid origin of DNA replication (*oriP*) of EBV, they can replicate as plasmids in these cells¹⁰. The lytic phase of the life cycle of the endogenous EBV was induced⁶ in resistant cells by TPA (20 ng ml⁻¹) and *n*-butyrate (3 mM), which also supports the amplification of the introduced p135 and p554 DNA. Four days after induction of the lytic cycle the cells and supernatants were collected. Virus was pelleted from the supernatants by ultracentrifugation and treated with DNase to destroy any non-encapsidated DNA. Cellular and viral DNA was isolated, digested with *Bam*HI, electrophoresed in 0.7% agarose gel, transferred to a nylon membrane and detected by hybridization with a radiolabelled probe of pKan2 (ref. 10). Molecular markers were a *Bst*EII digest of lambda DNA, but are not shown. Construct p554 was derived from p135: p135 contains the contiguous EBV DNA sequences of the B95-8 strain that lie between the *Eco*RI site at 7,315 and the *Sal*I site at 56,081



(ref. 20) inserted into the vector, pKan2 (ref. 10), but has only two copies of the *Bam*HI W fragment; p554 was derived from it by insertion of the region of terminal repeats of EBV found between an *Xho*I site at 169,423 and a *Sal*I site at 644 (ref. 20) into a unique *Nru*I site in p135 upstream of the TK promoter, within the pKan2 vector sequences¹⁰.

event between P3HR1 and p554 DNA. Those immortalized cells that contained the complementing DNA had no detectable recombinant molecules (Fig. 2c, clones 1 and 2). These findings indicate that a packaged, concatemeric derivative of p554 is infectious and that the deletion in the P3HR1 strain of EBV renders it non-immortalizing. The defective phenotype resulting from the deletion in the P3HR1 strain of EBV can be cured either by complementation or recombination.

The two genes in p554 that are deleted in P3HR1 were mutated to identify whether one or both of them are required for immortalization. Two derivatives of p554 were constructed. One contains termination codons positioned at one-third of the length of the *EBNA-2* open reading frame, p615 (*EBNA-LP*⁺, *EBNA-2*⁻); the other has the last two exons of *EBNA-LP* deleted, as they are in the P3HR1 strain, p633 (*EBNA-LP*⁻, *EBNA-2*⁺). These derivatives were introduced into the cell line containing the P3HR1 strain of EBV, the cells were then induced

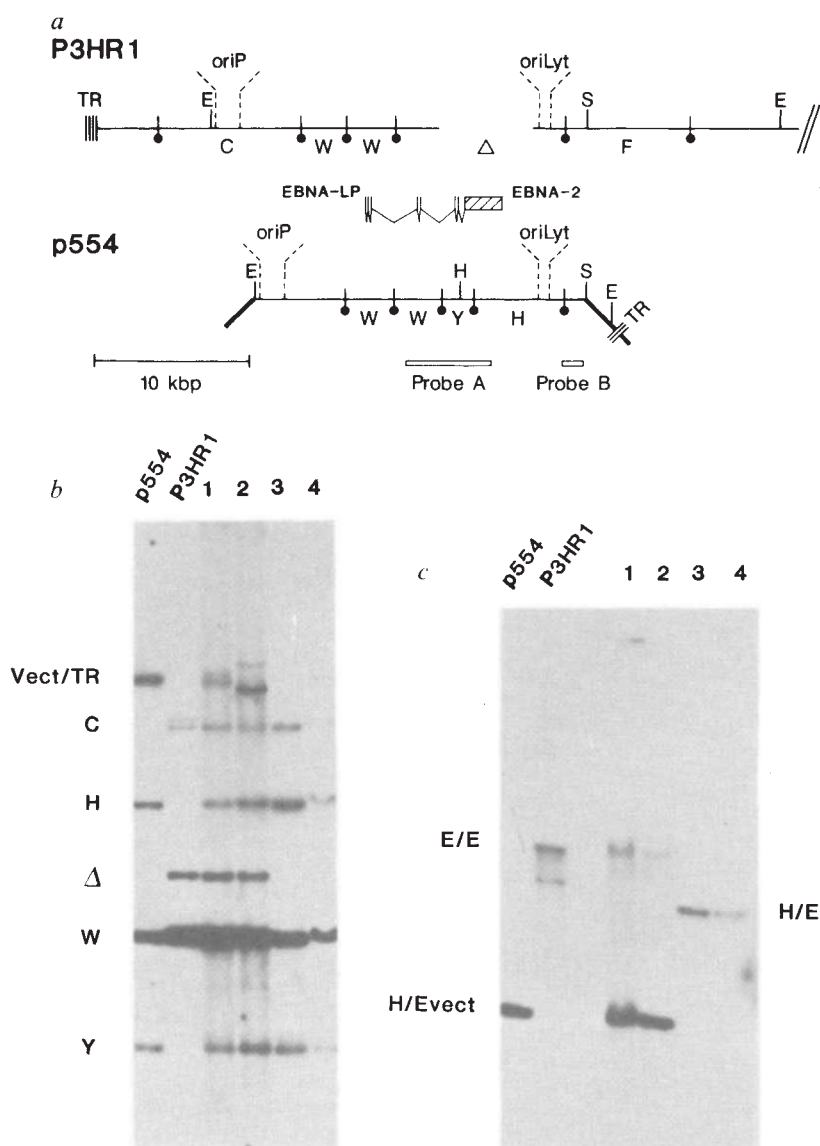
to support the lytic phase of the viral life cycle and the resulting viral stocks used to infect primary B lymphocytes. P3HR1 + p615 (*EBNA-LP*⁺, *EBNA-2*⁻) failed to immortalize B lymphocytes, whereas P3HR1 + p633 (*EBNA-LP*⁻, *EBNA-2*⁺) did (Table 1), although less efficiently than the virus derived from P3HR1 + p554 (Table 1).

The plasmid p615 (*EBNA-LP*⁺, *EBNA-2*⁻), when introduced into an established cell line, can express the amino terminal one-third of the *EBNA-2* open reading frame. The truncated protein was detected antigenically with a western blot of extracts of P3HR1 cells transfected with p615 (*EBNA-LP*⁺, *EBNA-2*⁻) (Fig. 3a). We therefore conclude that the expressed one-third of the *EBNA-2* gene is insufficient to immortalize human B lymphocytes and consequently that a functional *EBNA-2* gene product is required, presumably in conjunction with other viral genes, to immortalize B lymphocytes efficiently.

To confirm that viruses derived from P3HR1 + p633 (*EBNA-*

FIG. 2 Characterization of EBV-related DNA in B lymphocyte clones immortalized by a stock of P3HR1 + p554-derived virus. *a*, The structures of a linear form of P3HR1 viral DNA and the p554 plasmid DNA are shown. The vertical lines marked with *TR* represent the terminal repeats. The origins of plasmid and lytic replication of EBV are represented by *oriP* and *oriLyt* respectively. The gap in P3HR1 DNA denoted with a Δ indicates the deletion within P3HR1 DNA that removes the last two exons of *EBNA-LP* and all of *EBNA-2* open reading frame⁷. The bi-cistronic transcript of these two genes²¹ is depicted below the P3HR1 DNA, with the vertical lines (*EBNA-LP*) and hatched box (*EBNA-2*) representing its exons. The letters above the DNAs indicate sites of cleavage by different endonucleases: *E*, *EcoRI*; *S*, *Sall*; *H*, *HindIII*. The inverted lollipops indicate sites of cleavage by *Bam*HI, and the letters below the depicted DNA between the lollipops denote the names of different *Bam*HI cleavage products of EBV. The thickened lines in p554 represent vector sequences derived from pKan2 (ref. 10). The locations of the probes used for Southern blotting are denoted by open boxes. *b*, Isolated DNA was digested with *Bam*HI and analysed with probe A in pUC 18. The lanes contained p554 plasmid DNA (0.1 ng), strain HH514 clone 16 of P3HR1 (ref. 15) cellular DNA (0.2 μ g), and cellular DNA from clones 1 (2 μ g), 2 (2 μ g), 3 (0.2 μ g), and 4 (2 μ g). Vect/TR identifies signals from sequences with homology to pUC. In clones 1 and 2 these signals are heterogeneous because they include different numbers of copies of the terminal repeats, resulting from the cleavage within different repeats, that accompanies packaging (Fig. 1). C represents the *Bam*HI C fragment, which shares homology with the *Bam*HI W fragment²⁰. H, W, and Y, represent, respectively, the *Bam*HI H, W, and Y fragments. Δ represents the *Bam*HI fragment in P3HR1 DNA that contains the noted deletion. *c*, Isolated DNA was digested with *Eco*RI and *Hind*III and analysed with purified probe B (as shown in Fig. 2a). The lanes had the same DNA as in *b*. E/E represents the *Eco*RI-*Eco*RI fragment of > 30 kb from P3HR1 DNA (shown in Fig. 2a), which lacks an intervening *Hind*III site because of its deletion with P3HR1. H/E vect represents the 10 kb *Hind*III-*Eco*RI fragment in p554, in which the *Eco*RI site lies within prokaryotic vector sequences. H/E represents the *Hind*III-*Eco*RI fragment of ~ 20 kb in recombinant DNA in which the *Hind*III site is derived from p554 and the *Eco*RI site would be the right-most site depicted in P3HR1 DNA. No signal equivalent to H/E was detected in DNA of clones 1 and 2 at a sensitivity of one molecule per cell. The additional band in the unmarked P3HR1 lane most probably represents an uncharacterized defective EBV variant present in our HH514 strain of P3HR1 cells. This DNA is also seen in the P3HR1 lane in *b*, migrating just above the *Bam*HI C fragment. We have not found it in any immortalized clones.

METHODS. Clones of cells immortalized and noted in Table 1 were expanded, and total DNA was isolated from 5×10^7 cells. The virus-related DNA was



detected as in the legend to Fig. 1. Probe A is a *Dra*I fragment (nucleotides 44,768–50,305 of EBV (ref. 20)) in pUC, and probe B is an isolated *Bgl*II to *Sall* fragment (nucleotides 55,097–56,081 of EBV (ref. 20)). The gel in *b* contained 0.8% agarose, and that in *c* contained 0.4% agarose. Markers (not shown) in *b* were a *Hind*III digest of lambda and in *c* were a *Sty*I digest and a *Bst*EII digest of lambda DNA.

FIG. 3 Detection of the *EBNA-2* protein and its derivative expressed from different plasmids and endogenous EBV DNA. *a*, Total cell extracts of the GG68 clone 5 of P3HR1 (ref. 18) cells carrying the vectors p554 and p615 (p615 contains a termination codon in all three reading frames one-third of the distance into the *EBNA-2* open reading frame) were separated on an 8.5% polyacrylamide gel and electrophoretically transferred to nitrocellulose. A monospecific antiserum to *EBNA-2* was first used to detect the wild-type protein in the extract of P3HR1 containing p554, but not in the parental P3HR1 cells (Fig. 3*a*, and data not shown). A human serum was then used on the same blot to detect the truncated form of *EBNA-2* (Δ EBNA-2) encoded by p615. This human serum also detected the *EBNA-1* protein of EBV in both lanes. Note that a signal derived from the wild-type *EBNA-2* is not present in the P3HR1 + p615 lane. *b*, The *EBNA-2* protein was detected with a monospecific antiserum as in *a* in extracts of: 721 cells, a clone of B lymphocytes immortalized by the B95-8 strain of EBV; clone 5 cells which were immortalized by a stock of P3HR1 + p554-derived virus and contain recombinant EBV DNA; and a pool of B lymphocytes immortalized by a stock of P3HR1 + p633-derived virus (p633 is shown in Fig. 4).

METHODS. Extracts of 5×10^5 cells were analyzed as described²² by use of a rabbit monospecific antiserum to recombinant *EBNA-2* product (provided by Dr Georg Bornkamm), or by use of a human serum from an EBV-infected donor. Stained molecular weight markers were included in each gel (not shown). The apparent molecular weight of *EBNA-2* was 80,000–90,000 and 30,000–35,000 for the truncated *EBNA-2*.

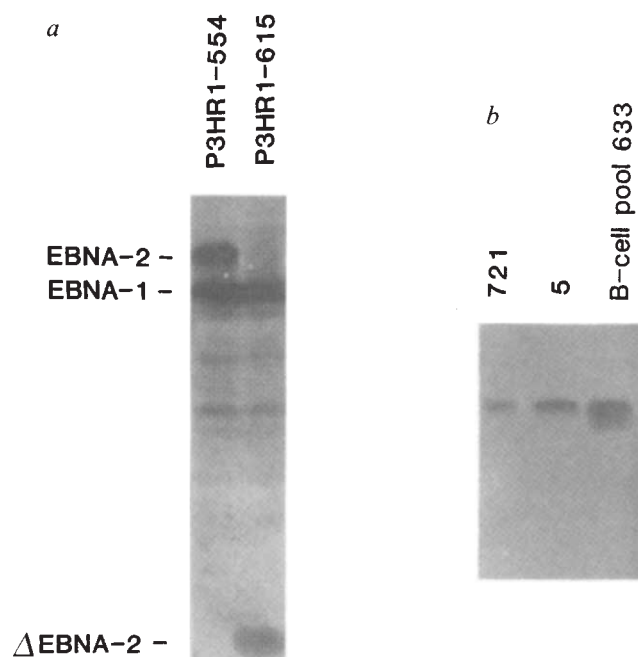
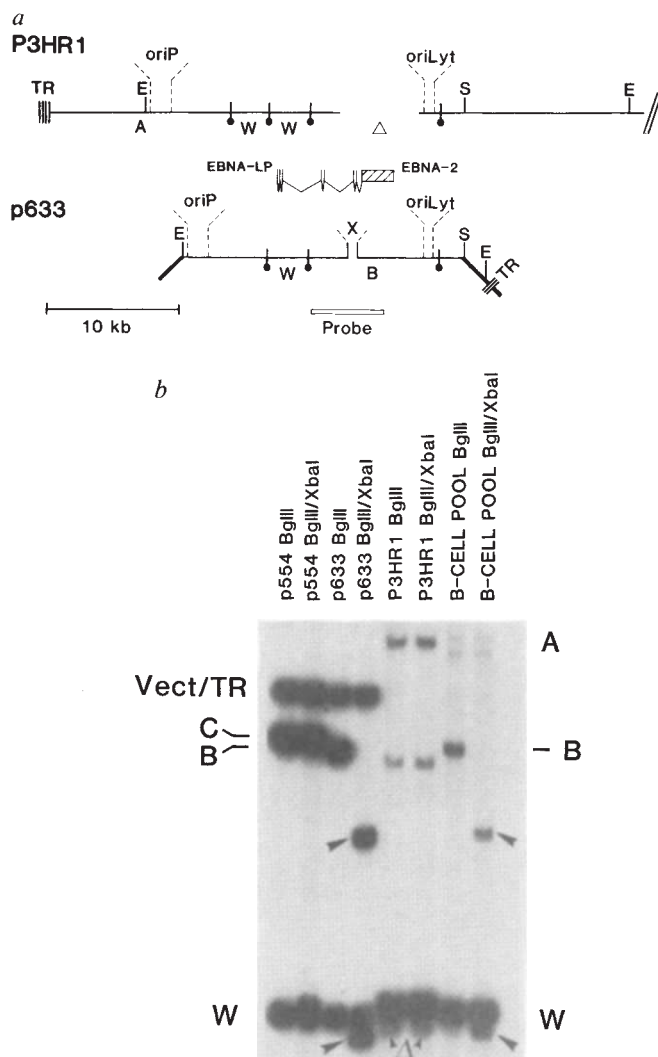


FIG. 4 Characterization of EBV-related DNA in a pool of cells immortalized by a stock of virus derived from P3HR1 + p633. *a*, The structures of a linear form of endogenous P3HR1 viral DNA and the p633 plasmid DNA are shown. The symbols used are equivalent to those in Fig. 2*a*, except that the inverted lollipops indicate sites of cleavage by *Bgl*III, and the letters between the inverted lollipops, below the depicted DNA, denote names of the different *Bgl*III cleavage products used in this study. The gap in p633 DNA marked with an X indicates the position of deletion of ~500 bp into which a diagnostic cleavage site for *Xba*I was introduced. This deletion removes the last two exons of *EBNA-LP*. *b*, Isolated DNA was digested with *Bgl*III or *Bgl*III plus *Xba*I and analyzed with probe A in pUC (see Fig. 2). The lanes contain the cleaved, plasmid DNA (1 ng) or cleaved cellular DNA isolated from the HH514 clone 16 strain of P3HR1 (0.8 μ g) or from a pool of cells immortalized by a stock of virus derived from the HH514 clone 16 strain of P3HR1 plus p633 (0.3 μ g). Vect/TR identifies the fragment that contains the terminal repeats in p554 and p633 DNAs detected by homology to pUC sequences. *B* represents the *Bgl*III fragment in p633, which contains the 500-bp deletion and the inserted, diagnostic *Xba*I cleavage site. An *Xba*I digestion of *Bgl*III cleaved plasmid p633 or DNA derived from the B cell pool containing this region of p633 cleaves the *Bgl*III B fragment to give the two fragments denoted with large, filled arrows in lanes 4 and 8. *C* represents a fragment in p554 equivalent to *B* in p633, except that it lacks the deletion and the *Xba*I site and is, therefore, 500 bp larger than *B*. *W* represents the *Bgl*III fragment equal in size to the *Bam*HI *W* fragment of EBV. Δ represents the *Bgl*III fragment of P3HR1 DNA, which contains the deletion encompassing part of *EBNA-LP* and all of *EBNA-2*. *A* represents the large *Bgl*III fragments that span the termini of EBV and are heterogeneous because they contain different numbers of terminal repeats. The fragment in the endogenous P3HR1 DNA migrating faster than *B* is derived from the defective EBV variant in the HH514 strain of P3HR1 cells. It has not been found in any cells immortalized *in vitro*.

METHODS. DNA was digested with *Bgl*III or *Bgl*III and *Xba*I. The DNA was then separated electrophoretically in a 0.7% agarose gel, transferred to nylon, and detected by hybridization to radiolabelled probe A in pUC of Fig. 2. A *Bst*EII digest of lambda DNA was included as a molecular weight marker (not shown). The vector p633 was derived from p554 by deletion of the sequences between an *Xho*I site and a unique *Hind*III site (nucleotides 47,526 to 48,039 of EBV (ref. 20)) and insertion of an oligonucleotide containing a *Xba*I recognition site at the point of the deletion.



LP⁻, EBNA-2⁺) were sufficient to immortalize human B lymphocytes, we propagated a pool of infected cells. These cells grew slowly and were propagated for 4 months before analysis for the expression of EBV gene products and the presence of viral DNA. These cells were found to express EBNA-2 (Fig. 3b). Their total DNA was extracted and P3HR1 and p633-derived DNA analysed by the method of Southern¹². Construct p633 was designed to permit easy identification by Southern analysis: its 500-bp deletion, encompassing the last two exons of EBNA-LP, was tagged with a synthetic *Xba*I recognition site (Fig. 4a). The viral DNA detected in the pool of B lymphocytes immortalized by virus derived from P3HR1 plus p633 does preserve the deletion characteristic of p633 and its unique *Xba*I site (Fig. 4b). This analysis indicates that the only viral DNA in the cells immortalized by virus derived from P3HR1 + p633 is a recombinant between these two DNAs. We conclude that the last two exons of the EBNA-LP gene are not required for immortalization of human B lymphocytes. It is possible, however, that an intact EBNA-LP gene could contribute to the growth characteristics of EBV-immortalized cells. It is also possible that, in *cis*, the deletion in p633 affects the expression of other viral genes, perhaps at the level of splicing, such that the altered expression affects the growth characteristics of cells immortalized by recombinants between P3HR1 and p633.

The finding that EBNA-2 is required for efficient immortalization of human B cells indicates that this gene could be used as a selectable insertional mutagen in the P3HR1 strain of EBV^{13,14}. We are now using this approach to identify additional immortalizing functions of EBV. The findings that DNA which contains *oriLyt* and the terminal repeats of EBV can be amplified, cleaved and packaged and that the packaged DNA

is infectious in primary human B lymphocytes should provide a route for efficiently introducing desired genes into these cells. Human B lymphocytes were traditionally considered refractory to the uptake of DNA. The use of an EBV virion coat can overcome this problem. □

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1. Miller, G. in *Virology* (ed. Fields, B. N.) 563-589 (Raven, New York, 1985).
2. Farrel, P. J., Speck, S., Knutson, J. & Gordon J. in *Advances in Viral Oncology* (ed. Klein, G.) 103-185 (Raven, New York), 1989).
3. Hammerschmidt, W. & Sugden, B. *Cell* **55**, 427-433 (1988).
4. Tamashiro, J. C. & Spector, D. H. *J. Virol.* **59**, 591-604 (1986).
5. Hammerschmidt, W. *et al. J. Virol.* **62**, 1355-1363 (1988).
6. zur Hausen, H., O'Neill, F. J. & Freese, U. K. *Nature* **272**, 373-375 (1978).
7. Jeang, K.-T. & Hayward, S. D. *J. Virol.* **48**, 135-148 (1983).
8. Rabson, M., Gradoville, L., Heston, L. & Miller, G. *J. Virol.* **44**, 834-844 (1982).
9. Countryman, J. & Miller, G. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4085-4089 (1985).
10. Yates, J., Warren, N., Reisman, D. & Sugden, B. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3806-3810 (1984).
11. Yates, J., Warren, N. & Sugden, B. *Nature* **313**, 812-815 (1985).
12. Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
13. Smiley, J. R. *Nature* **285**, 333-335 (1980).
14. Post, L. E., Mackem, S. & Roizman, B. *Cell* **24**, 555-565 (1981).
15. Rabson, M., Heston, L. & Miller, G. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2762-2766 (1983).
16. Magee, M. G., McHugh, L. & Rothstein, T. *J. Immunol. Meth.* **15**, 47-56 (1977).
17. Sugden, B. & Mark, W. *J. Virol.* **23**, 503-508 (1977).
18. Heston, L., Rabson, M. S., Brown, N. & Miller, G. *Nature* **295**, 160-163 (1982).
19. Knutson, J. C. & Yee, D. *Analyt. Biochem.* **164**, 44-52 (1987).
20. Baer, R. *et al. Nature* **310**, 207-211 (1984).
21. Wang, F., Petti, L., Braun, D., Seung, S. & Kieff, E. *J. Virol.* **61**, 945-954 (1987).
22. Balchwal, V. R. & Sugden, B. *J. Virol.* **61**, 866-875 (1987).

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Complete mutagenesis of the HIV-1 protease

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RETROVIRUSES encode a protease which needs to be active for the production of infectious virions. A disabling mutation in the protease results in the production of non-infectious virus particles and examination of proteins from these mutant virions reveals unprocessed Gag and Gag-Pol precursor proteins, the substrates of the viral protease¹⁻³. Each amino acid of the HIV-1 protease was individually mutated using a simple mutagenesis procedure which is capable of introducing and identifying missense mutations in each residue of a protein. Phenotypic screening of these mutants in a heterologous assay system reveals three regions within the protease where multiple consecutive amino-acid residues are sensitive to mutation. These results show that random mutagenesis can be used to identify functionally important regions within a protein. Mutants with conditional phenotypes have also been identified within this collection.

The HIV-1 protease is encoded within the viral *pol* gene and is enzymatically active when expressed in *Escherichia coli* either as the larger precursor form⁴⁻¹⁰ or just as the protease domain^{3,11}. When the protease is expressed linked to the entire Pol protein domain, the processed forms of the *pol* gene products are generated (ref. 4, 6, 9, 10 and Fig. 1). The HIV-1 *pol* gene encodes five proteins: a protease (p11, PR); two forms of the reverse transcriptase (p64 and p51; RT); RNase H (p13, RN),

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TABLE 1 Immortalization of human B lymphocytes by packaged, engineered DNA derived from EBV and immortalization-incompetent helper viruses

Virus inoculum*	Number of proliferating cell clones† per 10 ⁶ infected B lymphocytes
1 ml P3HR1 (EBNA-LP ⁻ , EBNA-2 ⁻)	0, 0
3 ml P3HR1	0, 0
5 ml P3HR1	0, 0, 0, 0, 0, 0
1 ml P3HR1 + p544 (EBNA-LP ⁺ , EBNA-2 ⁺)	30, 80, 20
3 ml P3HR1 + p554	220, 270
5 ml P3HR1 + p554	650, 480, 240, 800, 1,020, 370, 100
5 ml P3HR1 + p615‡ (EBNA-LP ⁺ , EBNA-2 ⁻)	0, 0
5 ml P3HR1 + p633§ (EBNA-LP ⁻ , EBNA-2 ⁺)	30, 40

* Virus stocks were collected by induction of the HH514 clone 16 strain of P3HR (ref. 15) cells by introduction of pCMV BZLF-1 (see ref. 3) into the cells by electroporation or by co-introduction of both p554 and pCMV BZLF-1 (ref. 3); 10 µg each vector was introduced per 10⁷ cells. Five days following electroporation, cell supernatants were collected, filtered through 0.2-micron filters, and stored at 4 °C until used for infection.

† B lymphocytes were isolated from peripheral blood and purified by panning with anti-human immunoglobulin as described¹⁶. The B lymphocytes, while still bound to the plates, were exposed to virus overnight, detached from the plates by pipetting and distributed in agarose over a human fibroblast feeder layer as described¹⁷. Colonies were scored 4-6 weeks later (each number represents an independent infection) and fed every 10-14 days as described¹⁷. Colonies were picked and expanded on lethally irradiated monolayers of human fibroblasts for further analysis.

‡ The structure of the plasmid p615 is the same as that of p554 (Fig. 2a), except that it contains termination codons in all three reading frames at a *Bst*II site one-third of the distance into the EBNA-2 open reading frame.

§ The structure of the vector p633 is shown in Fig. 4a. It contains a deletion compared with p554 (Fig. 2a), which removes the last two exons of the EBNA-LP open reading frame and leaves the open reading frame of EBNA-2 intact.

and the integrase (p34, IN). When expressed in *E. coli*, these viral proteins can be detected by western blot analysis of the bacterial extract with an appropriate antiserum. In our standard assay, we use an antiserum¹² that can detect the two forms of the RT, p64 and p51, and the PR, p11 (Fig. 1, compare lanes V and 1). Mutations that block Pol protein processing make what seems to be the primary translation product and no mature products (Fig. 1, lane 3). Some mutants have an intermediate phenotype with the presence of mature products in addition to some high molecular weight products (Fig. 1, lane 2). We have used this assay to determine the phenotype of more than 900 clones containing missense mutations in the HIV-1 protease. Approximately 1,700 clones were sequenced resulting in the identification of clones containing over 330 different single missense mutations that included at least one substitution in each

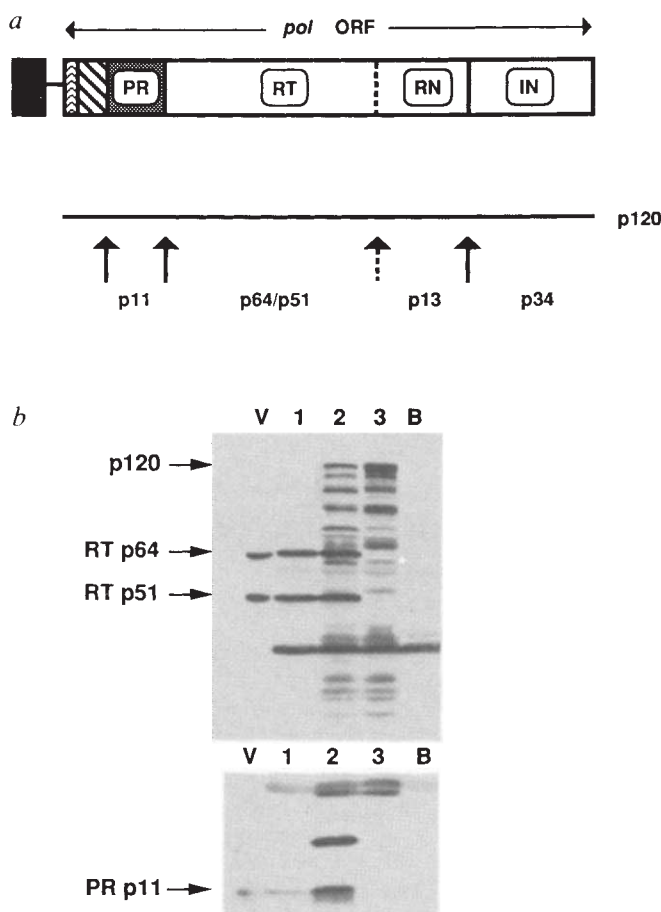


FIG. 1 *a*, Structure of the HIV-1 *pol* gene in the expression clone pART2 (ref. 6) and its associated translation products. Rectangular box, *pol* open reading frame (ORF), with specific domains. PR, protease; RT, reverse transcriptase; RN, RNase H; IN, integrase. The two domains upstream of the PR domain represent sequences contributed by the vector pIBI20 (23 amino acids) and upstream *pol* gene (52 amino acids). Solid black box, the *E. coli* lac PO Line labelled p120, primary translation product of pART2. Vertical arrows, protease cleavage sites, with mature products labelled underneath. Broken-line arrow, partial cleavage at this site to generate submolar amounts of p51 RT. *b*, Two western analyses demonstrating the three phenotypic categories of HIV-1 protease mutants. Lanes: V, lysate of pelleted HIV-1; 1, lysate of bacteria expressing a wild-type phenotype protease; 2, lysate of bacteria expressing protease with an intermediate phenotype; 3, lysate of bacteria expressing protease with a negative phenotype; B, lysate of bacteria expressing pIBI20, the parent of pART2, which does not contain HIV-1 sequences. The upper panel is from a 10% polyacrylamide gel and the lower panel is from a 15% polyacrylamide gel. Both panels were stained with a polyclonal rabbit antiserum directed against the C terminus of the HIV-1 PR and the N terminus of the HIV-1 RT (ref. 12).

of the 99 amino acid residues of the HIV-1 protease. This collection represents ~18% of all possible single missense substitutions and ~50% of the missense substitutions obtainable by single nucleotide changes.

Mutant proteases were placed into three phenotypic categories based on their ability to process the Pol precursor in *E. coli*: (1) wild-type or positive—processed similarly to the wild-type clone as indicated by the presence of mature Pol products; (2) negative—no mature processed products and instead what appeared to be the primary translation product; (3) intermediate—some mature products but also some high molecular weight products. The intermediate phenotype contains the widest variety of phenotypes with the ratio of mature products to unprocessed product being variable. Figure 2a is a summary of the phenotypes associated with the single missense substitutions of the HIV-1 protease.

The data set presented in Figure 2a can be analysed on several different levels. One approach is to work with the entire data set to obtain a broad view of how the protease is functionally organized. To determine the sensitivity of regions of the HIV-1 protease to mutagenesis, we constructed a histogram of residue position versus mutant protease activity (Fig. 2b). In this analysis, only non-conservative substitutions are considered. If a residue is able to tolerate a non-conservative substitution and still retain protease activity, the chemical nature of the side chain is probably unimportant for protease function. Conversely, if a residue is consistently sensitive to mutational inactivation it may play a key role in protein function. In Figure 2b the height of the bar is proportional to the level of protease activity. If an amino-acid position had multiple phenotypes on substitution, the most active phenotype is noted.

This analysis reveals three noncontiguous regions having high mutational sensitivity. The first region (residues 22 to 33) includes the sequence D25 T26 G27 which has strong similarity to the active site of aspartic proteinases¹³, where D25 is equivalent to the catalytically active aspartic acid residue. The second region is a short glycine-rich sequence (IGGIGG, residues 47 to 52) in the centre of the protease sequence. The third region is the largest, 14 residues in length (T74 to R87). It is generally hydrophobic and bisected at position V82 by a less-sensitive residue. This mutationally sensitive region may extend to position 90.

The observation that regions of consecutive amino acids are sensitive to mutation suggests two features concerning the protease. First, the number of sensitive amino acids is large relative to the length of the protein (40%). Because the number of amino acids directly involved in catalysis would be expected to be small, this suggests that most mutations are destroying enzyme function by altering structural determinants. Shortle and Lin¹⁴ have made a similar proposal based on an analysis of mutants of staphylococcal nuclease, as have Pakula *et al.*¹⁵ based on mutagenesis of the Cro repressor. Second, the fact that most of the mutationally sensitive amino acids appear in blocks suggests that these amino acids provide features of protein structure distinct from those provided by mutationally sensitive amino acids outside the blocks. One possibility is that the participation of consecutive amino acids in a structural domain is required to function in a cooperative way to provide the necessary rigidity to the conformation of the active site.

Greater detail about the role of these regions can be inferred by examining their location in the recently described structure of the HIV-1 protease¹⁶. Shown in Figure 3 is a representation of one subunit of the protease homodimer. The dimer shows structural elements of aspartic proteinases in having two aspartic acid residues (D25 from each subunit) juxtaposed to an area that seems to be the substrate binding cleft. This cleft is covered by a flap that in aspartic proteinases can interact with substrate and there are additional conserved structural elements within each monomer. The first mutationally sensitive region (A22–L33) forms a loop which runs along the presumed substrate-

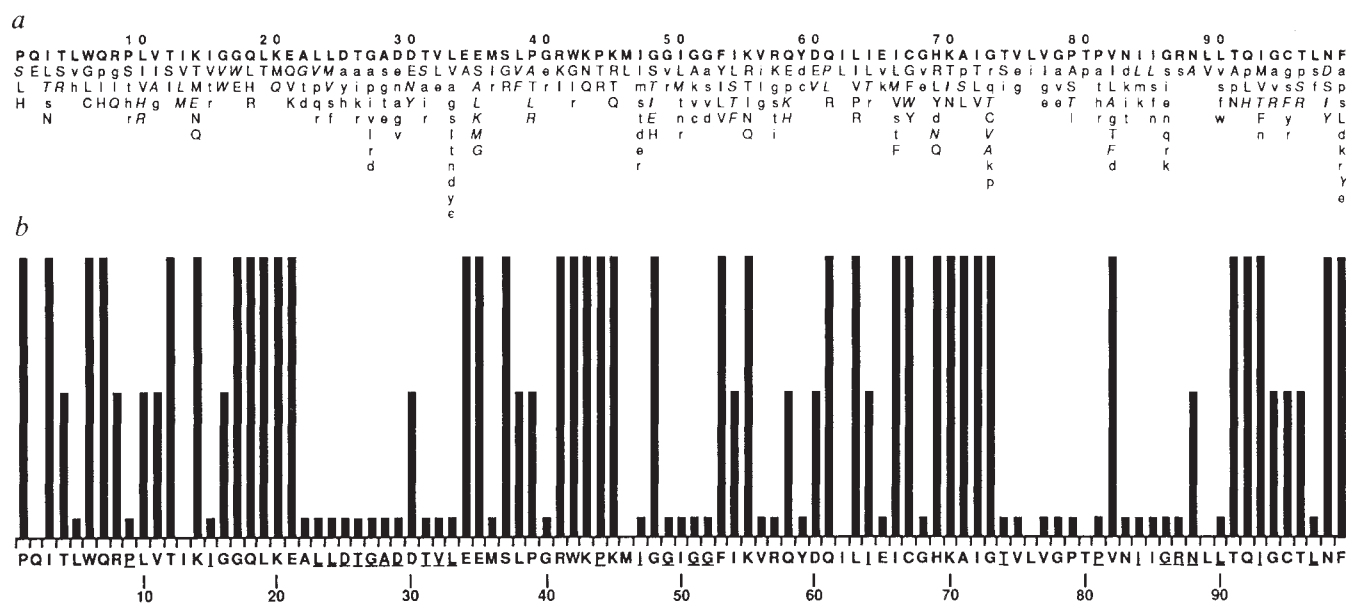
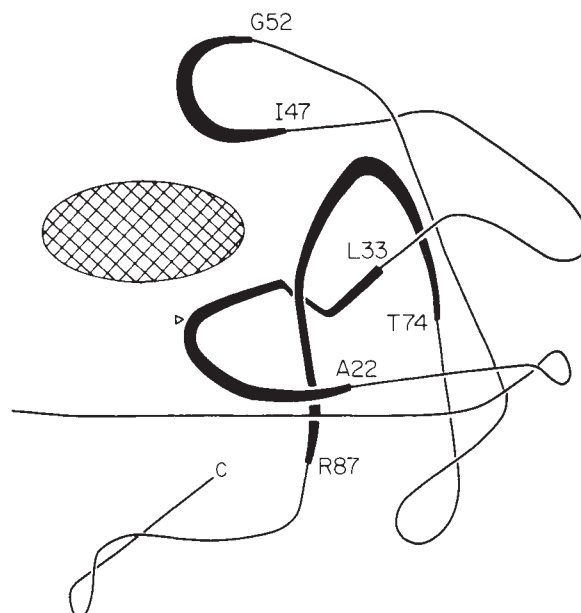


FIG. 2 *a*, Single missense mutations and their phenotypes. The top horizontal line of bold upper-case letters is the amino-acid sequence of the HIV-1 protease, in single-letter code. Residues are numbered every 10 positions. Vertical column beneath each residue, single missense mutations tested at that position. The phenotypes are encoded as follows: upper-case letter, wild-type or positive phenotype; italicized upper-case letter, intermediate phenotype; lower-case letter, negative phenotype. A positive or intermediate isoleucine is indicated by a bold uppercase I to distinguish it from a negative leucine. *b*, Protease activity associated with nonconservative amino acid substitutions. Horizontal line of letters, amino-acid sequence of the HIV-1 protease. Underlined positions, conserved residues in an alignment of different retroviral protease amino acid sequences⁶. Vertical black bars above each position, effect of a non-conservative amino-acid substitution at that position on the ability of the protease to process. Tallest bar, wild-type or positive activity; smallest bar, negative activity; intermediate-height bars, intermediate protease activity. When multiple phenotypes were recorded at one position, the most active phenotype is noted. The non-conservative amino-acid groups used in this analysis are as follows: A, S, T, G and P; V, L, I and M; F, Y and W; R, K and H; D, E, N and Q; and C (ref. 23). **METHODS.** To carry out the mutagenesis, 8 mutagenic oligonucleotides (39–46 bp), which covered the 297-bp protease domain of the *pol* gene, were synthesized under conditions such that an average of 1.5 mutations per oligonucleotide were incorporated²⁰. The mutagenic oligonucleotides

were used individually to create libraries of random point-mutations by using the oligonucleotides as primers for DNA synthesis with a uracil-containing, single-stranded DNA form of pART2 template following the procedure of Kunkel *et al.*²¹ with the following modifications. The mutagenic oligonucleotide to template DNA molar ratio used in the annealing reaction was between 0.5 and 1. A 3:1 mass ratio of *E. coli* single-strand binding protein to template DNA was used in the *in vitro* DNA synthesis reaction to promote DNA synthesis. Libraries of mutant clones were generated by transformation of *E. coli* JM101 with 100–200 ng DNA from the synthesis reaction. This typically generated 50,000–100,000 independent ampicillin-resistant colonies. These resultant libraries had >50% of the clones containing at least one nucleotide substitution. These libraries were packaged into filamentous particles²² before re-introduction into fresh *E. coli* JM101 to prevent analysing clones containing mixed genotypes. Phenotype and genotype determinations were carried out as described by Loeb *et al.*⁶. At nine positions (residues 27, 33, 35, 47, 48, 51, 73, 86 and 99) an oligonucleotide was synthesized in which all three positions for that codon had an equimolar mixture of all four nucleotides. Separate libraries were made with each of these oligonucleotides using the procedure described above. Data from two libraries (M, Q) that have been described previously⁶ are included, with the following two corrections. A28 to S, which was scored intermediate on the basis of a very low level of processing, has been scored negative upon further study; and K43 to E is corrected to K43 to Q.

FIG. 3 Placement of the mutationally sensitive domains on the HIV-1 protease structure. The diagram is a smooth line tracing of the HIV-1 monomer structure¹⁶. C terminus is denoted by letter C. The N terminus seems to be disordered in the crystal structure¹⁶. The first N-terminal amino acid shown is residue 6. The small triangle indicates the location of catalytic aspartic-acid residue (residue 25). Cross-hatched oval, probable location of the substrate in the active site; thicker-lined regions in the chain, three mutationally sensitive domains, with the width of the line indicating perspective. Residues and positions at the beginning and end of each mutationally sensitive domain are indicated.



binding cleft and then turns into the core of the molecule. There the chain passes through a loop formed by the large C-terminal region (T74–R87). These features represent the bulk of the mutationally sensitive amino acids and presumably the main structural and functional determinants of the protein. This structural feature of the protein chain just beyond the active site passing through a loop in the interior of the enzyme can be seen as a conserved element in other aspartic proteinases and could constitute the basic structural feature of these enzymes^{17,18,19}.

The third sensitive region is in the flap. The sensitivity of this region suggests it is also important for the viral protease even though it is placed topologically distal to the other sensitive regions. The activity of one conditional mutant suggest that this flap region also interacts with substrate for the viral protease.

Several mutations in the mutationally sensitive central region resulted in an altered processing profile which was distinct from the typical intermediate phenotype. One mutant (Gly 48 → Glu) shows a phenotype in which there is a reduced amount of p51 such that the ratio of p64 to p51 is increased (Fig. 4, lanes 1, 2). One explanation is that the cleavage of p64 leading to p51 is reduced. Because the various processing sites in the HIV-1 Gag and Pol precursors do not have the same sequence, the protease might not interact with them equivalently. The above mutant may not interact with the processing site that generates the C terminus of p51 as well as the wild-type protease, leading to the observed phenotype.

We have begun to screen the non-wild-type mutants, which were initially scored at 37 °C, for their ability to process at 32 °C. Most of the mutants do not show any difference in their ability to process at the lower temperature. The few which do show activity at 32 °C are intermediate in their processing ability. One example is a Pro → Thr substitution at position 79 (Fig. 4, lanes

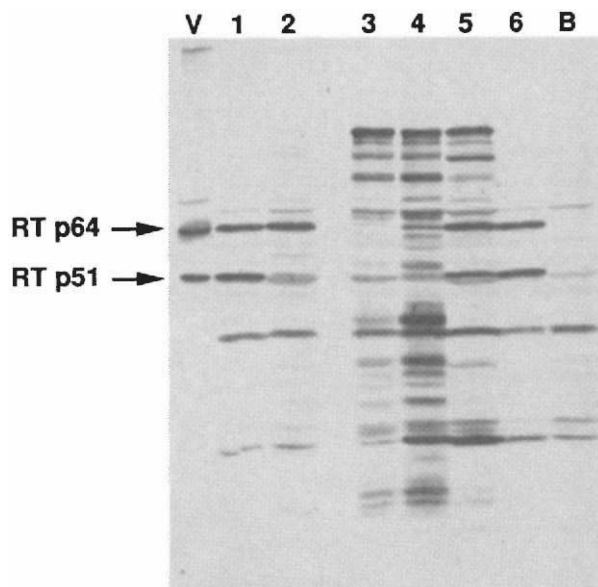


FIG. 4 Western blot analysis of examples of conditional mutants. Lanes: V, lysate of pelleted HIV-1; 1, lysate of bacteria expressing the wild-type clone pART2; 2, lysate of bacteria expressing pART2 with a G48 to E mutation in PR; 3, lysate of bacteria expressing pART2 with a mutation that gives a negative protease phenotype; 4, lysate of bacteria grown at 37 °C expressing pART2 with a P79 to T mutation in PR; 5, lysate of bacteria grown at 32 °C expressing a P79 to T mutation in PR; 6, lysate of bacteria expressing the wild-type clone pART2; B, lysate of bacteria expressing pIBI20, the parent of PART2, which does not contain HIV-1 sequences. The antibody used in this experiment was a rabbit anti-serum directed against a synthetic peptide corresponding to residues 9 to 22 of the mature RT. Peptide synthesis and preparation of polyclonal anti-peptide antiserum was carried out as described by Dewey *et al.*²⁴ except for the use of succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate as the conjugating agent.

4 and 5). This mutant, initially scored as an intermediate phenotype at 37 °C, shows an increased ratio of mature products to precursor at 32 °C. Position 79 is in the turn of the large loop formed by the C-terminal mutationally sensitive region. Threonine at this position may reduce the probability of proper folding or reduce the stability of the folded structure.

The ability to generate missense mutation maps of many consecutive residues in a protein should aid our understanding of protein function and reveal conditional mutants in the absence of selection. A description of protein function should be able to synthesize the understandings derived from large-scale mutational analysis and other approaches such as structure determination and biochemical analysis to arrive at a more comprehensive understanding of protein function. □

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1. Crawford, S. & Goff, S. P. *J. Virol.* **53**, 899–907 (1985).
2. Katoh, I. *et al. Virology* **145**, 280–292 (1985).
3. Kohl, N. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 4686–4690 (1988).
4. Farmerie, W. G. *et al. Science* **236**, 305–308 (1987).
5. Debouck, C. *et al. Proc. natn. Acad. Sci. U.S.A.* **84**, 8903–8906 (1987).
6. Loeb, D. D., Hutchison, C. A. III, Edgell, M. H., Farmerie, W. G. & Swanstrom, R. *J. Virol.* **63**, 111–121 (1989).
7. Krausslich, H.-G., Scheidner, H., Zybarch, G., Carter, C. A. & Wimmer, E. *J. Virol.* **62**, 4393–4397 (1988).
8. Seelmeier, S., Schmidt, H., Turk, V. & von der Helm, K. *Proc. natn. Acad. Sci. U.S.A.* **85**, 6612–6616 (1988).
9. Mous, J., Heimer, E. P. & Le Grice, S. F. *J. Virol.* **62**, 1433–1436 (1988).
10. Hansen, J., Schulze, T. & Moelling, K. *J. biol. Chem.* **262**, 12393–12396 (1987).
11. Graves, M. C., Lim, J. J., Heimer, E. P. & Kramer, R. *Proc. natn. Acad. Sci. U.S.A.* **85**, 2449–2453 (1988).
12. Barr, P. J., Power, M. D., Lee-Ng, H. L., Gibson, H. L. & Luciw, P. A. *Bio/Technology* **5**, 486–489 (1987).
13. Toh, H. *et al. EMBO J.* **4**, 1267–1272 (1985).
14. Navia, M. A. *et al. Nature* **337**, 615–620 (1989).
15. Shortle, D. & Lin, B. *Genetics* **110**, 539–555 (1985).
16. Pakula, A. A., Young, V. B. & Sauer, R. T. *Proc. natn. Acad. Sci. U.S.A.* **83**, 8829–8833 (1986).
17. Subramanian, E. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 556–559 (1977).
18. James, M. N. G. & Sietlecki, A. R. *J. molec. Biol.* **163**, 299–361 (1983).
19. Andreeva, N. S., Zdanov, A. S., Gustchina, A. E. & Federov, A. A. *J. biol. Chem.* **259**, 11353–11365 (1984).
20. Hutchison, C. A. III, Nordeen, S. K., Vogt, K. & Edgell, M. H. *Proc. natn. Acad. Sci. U.S.A.* **84**, 710–714 (1986).
21. Kunkel, T. A., Roberts, J. D. & Zakour, R. A. *Meth. Enzym.* **154**, 367–382 (1987).
22. Vieira, J. & Messing, J. *Meth. Enzym.* **153**, 3–11 (1987).
23. Dayhoff, M. O., Schwartz, R. M. & Orcutt, B. C. in *Atlas of Protein Sequence and Structure* (ed. Dayhoff, M. O.) **5**, 345–352 (Nat. Biomedical Research Fdn, Washington, DC, 1978).
24. Dewey, R. E., Timothy, D. H. & Levings, C.S. III *Proc. natn. Acad. Sci. U.S.A.* **84**, 5347–5378 (1987).

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The yeast *STE6* gene encodes a homologue of the mammalian multidrug resistance P-glycoprotein

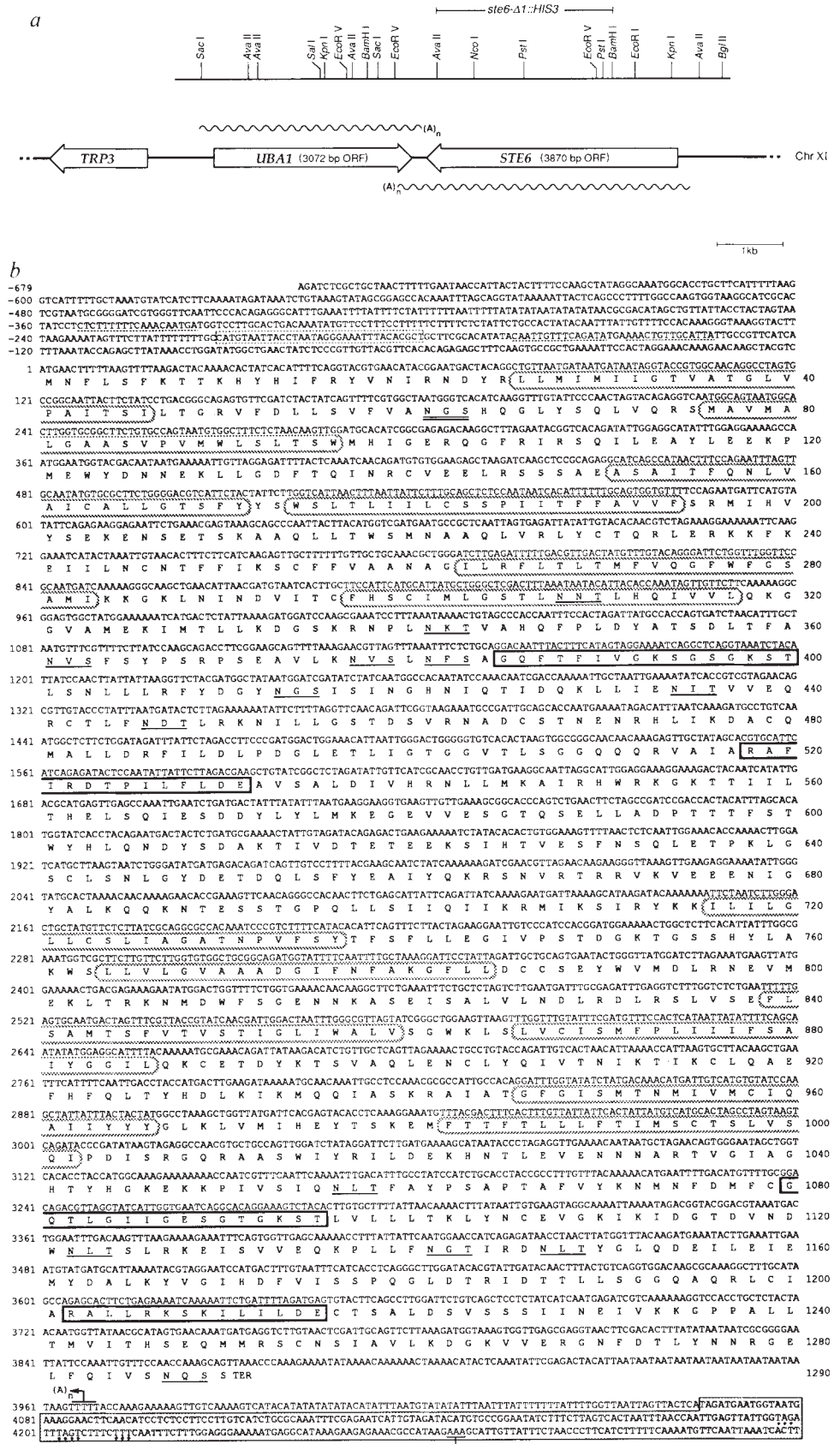
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MAMMALIAN tumours displaying multidrug resistance overexpress a plasma membrane protein (P-glycoprotein), which is encoded by the *MDR1* gene¹ and apparently functions as an energy-dependent drug efflux pump. Tissue-specific expression of *MDR1* and other members of the *MDR* gene family has been observed in normal cells², suggesting a role for P-glycoproteins in secretion. We have isolated a gene from the yeast *Saccharomyces cerevisiae* that encodes a protein very similar to mammalian P-glycoproteins. Deletion of this gene resulted in sterility of *MATa*, but not of *MATα* cells. Subsequent analysis revealed that the yeast P-glycoprotein is the product of the *STE6* gene, a locus previously shown to be required in *MATa* cells for production of

FIG. 1 Structure of the region of chromosome XI from *S. cerevisiae* containing *TRP3*, *UBA1* and the 3'-overlapping transcription unit (*STE6*). **a**, Restriction map of a ~9 kb region that contains the *UBA1* gene, which encodes the ubiquitin-activating enzyme, and a 3'-adjacent open reading frame (ORF), later identified as the *STE6* gene. Open arrows delineate the protein-coding regions. *UBA1* and *STE6* give rise, respectively, to ~3.2 kb and ~4.3 kb mRNAs which overlap by ~300 nucleotides at their 3' ends. Wavy lines indicate the spans of the two transcription units, mapped by sequencing a number of the corresponding cDNA clones. In the *ste6-Δ1::HIS3* deletion allele, a 2.7 kb *Bam*HI–*Ava*II fragment from within the *STE6* coding region was replaced, as shown, by the *HIS3* marker gene. **b**, Nucleotide sequence of *STE6* and deduced amino-acid sequence (one-letter code) of the *STE6* protein. Upstream transcriptional control elements in *STE6* include the binding site for $\alpha 2$ repressor²⁶ (dashed box), and sites involved in the control of *STE6* by α -factor²⁷ (dashed underlines). Nucleotide sequences resembling the tripartite consensus sequence for transcription termination in yeast²⁸ are marked by dots. The *STE6* cDNA clones extend over 200 bp into the coding region of the *UBA1* gene (shown within the shaded box) where they terminate with a poly(dA) tract, indicated by [(A)_n]. The polyadenylation site of the *UBA1* mRNA transcripts, which is located between the two coding regions, is indicated similarly. In the *STE6* amino-acid sequence, potential N-linked glycosylation sites (Asn-X-Ser/Thr) are underlined. As with all P-glycoproteins sequenced to date, only the first of the predicted extracytoplasmic segments of the *STE6* protein contains a potential N-linked glycosylation site (doubly underlined). The 12 predicted transmembrane segments are enclosed in boxes with rounded ends¹¹. Thick-bordered boxes enclose the amino-acid sequences which fit the consensus for a nucleotide binding site¹².

METHODS. The initial isolation of yeast genomic DNA clones, carried in λ gt11 and containing the *UBA1* gene (which encodes the ubiquitin-activating enzyme) will be described elsewhere. The yeast DNA insert from one of these clones that contained the 3' end of *UBA1* was used to screen both a yeast genomic DNA library in EMBL3A and a λ gt10-based yeast cDNA library prepared from poly(A)⁺ RNA isolated from stationary-phase cells of *S. cerevisiae* strain S288C. The *UBA1* gene was localized to chromosome XI by hybridization to an agarose gel containing intact *S. cerevisiae* chromosomal DNA separated by pulsed-field electrophoresis (Clontech Laboratories) (unpublished results). In addition, the nucleotide sequence upstream of the *UBA1* gene was found to be identical to the 5'-flanking sequence of the *TRP3* gene²⁹, which has previously been mapped to the left arm of chromosome XI (ref. 30). Nucleotide sequences were determined by the chain-termination method, using a



modified form of T7 DNA polymerase (Sequenase; United States Biochemical Corp.), after subcloning DNA fragments into M13 vectors and generating a series of overlapping deletions³¹.

a-factor pheromone³. Our findings suggest that the *STE6* protein functions to export the hydrophobic a-factor lipopeptide in a manner analogous to the efflux of hydrophobic cytotoxic drugs catalysed by the related mammalian P-glycoprotein. Thus, the evolutionarily conserved family of *MDR*-like genes, including the *hlyB* gene⁴ of *Escherichia coli* and the *STE6* gene of *S. cerevisiae*, encodes components of secretory pathways distinct from the classical, signal sequence-dependent protein translocation system.

The yeast P-glycoprotein homologue was discovered in the course of our work on the *UBA1* gene, which encodes the ubiquitin-activating enzyme⁵. Analysis of complementary DNA clones containing *UBA1* sequences revealed a convergently transcribed, 3'-adjacent locus whose transcripts overlap those of *UBA1* by ~300 nucleotides (Fig. 1a). The extent of overlap suggested that there could be regulatory interaction between *UBA1* and this gene, prompting us to further characterize it.

The organization of these overlapping transcription units on yeast chromosome XI is shown in Fig. 1a. The DNA sequence downstream of the *UBA1* gene contains a large open reading frame (ORF) in the opposite DNA strand that is separated from the *UBA1* coding region by 194 base pairs (bp) (Fig. 1b). A search of the sequence databases using the deduced amino-acid sequence of this downstream ORF (Fig. 1b) detected significant similarities to several bacterial transport proteins. The greatest similarity was to the *E. coli hlyB* gene product, a membrane protein required for the *secA*-independent translocation of α -haemolysin (a protein of relative molecular mass ~107,000) across the bacterial double membrane⁶. The yeast protein was also found to be homologous to the bacterial PstB, HisP, MalK, and OppD proteins, all of which are membrane-associated components of specific bacterial permeases⁷. These bacterial proteins have been previously found to share sequence similarities with the mammalian P-glycoprotein, whose functions include the mediation of multidrug resistance^{8,9}.

Comparison of the products of the human *MDR1* gene and the yeast ORF clearly identifies the *S. cerevisiae* protein as a homologue of the human P-glycoprotein. The *MDR1* gene and the yeast ORF encode, respectively, a 1,280-residue and a 1,290-residue protein. A graphic matrix analysis (Fig. 2a) features a nearly continuous line along the main diagonal, indicating extensive sequence similarity between the human and yeast proteins throughout their lengths. At the amino-acid sequence level, 57% of the residues are either identical or highly conserved¹⁰ between the two proteins. The two offset diagonals in

Fig. 2a indicate a tandem duplication in both proteins, reflecting their conserved dimer-like structure. This conservation of structural features between the human and yeast P-glycoproteins is most evident in their hydropathy profiles, which are nearly superimposable (Fig. 2b). Each half-molecule consists of an extensive hydrophobic region containing six putative transmembrane domains¹¹ arranged in pairs, followed by a hydrophilic domain containing the consensus sequences for a potential ATP-binding site¹² (Fig. 1b).

Having found the highly conserved P-glycoprotein in *S. cerevisiae*, we began to explore its function. To determine whether the yeast *MDR1* homologue is an essential gene, we constructed a deletion allele in which ~70% of the coding sequence was replaced with the *HIS3* marker gene (Fig. 1a), and introduced this mutation into the diploid DF5 strain¹³ by homologous recombination. When diploid His⁺ transformants, heterozygous for the deleted P-glycoprotein gene, were sporulated and subjected to tetrad analysis, most of the asci (26 of 29) yielded four viable spores. Southern blot analysis of DNA from individual segregants showed that the His⁺ phenotype cosegregated with the deletion (data not shown). Thus, the loss of the yeast P-glycoprotein gene has no effect on cell viability.

Subsequent analysis of the haploid strains lacking the P-glycoprotein gene uncovered a mating defect manifested only in cells of the a mating type. When tetrads from the gene deletion experiment were analysed for mating type, we found that all of the *MATa* segregants were mating-proficient, whereas the deletion-bearing segregants expected to be *MATa* were incapable of mating. Thus, the null phenotype of the P-glycoprotein gene is an a-specific sterility.

At least seven loci are required for mating specifically by a-cells: *STE2*, *STE6*, *STE14*, *STE16(RAM)*, *MFA1*, *MFA2*, and *ARD1* (ref. 14). Several of these genes were previously cloned and analysed, allowing direct comparisons with the *S. cerevisiae* P-glycoprotein gene. On examination, the published restriction map of the *STE6* gene³ was found to be identical to that of the P-glycoprotein gene. In addition, the only reported DNA sequence from the *STE6* gene, its promoter region¹⁵, is identical to the sequence upstream of the P-glycoprotein gene (Fig. 1b). We conclude that the yeast P-glycoprotein homologue is encoded by the *STE6* gene, a finding with immediate implications for the role of *STE6* in a-factor production.

Three of the a-specific sterile genes, *STE6*, *STE14* and *STE16(RAM)*, appear to function at the level of post-transla-

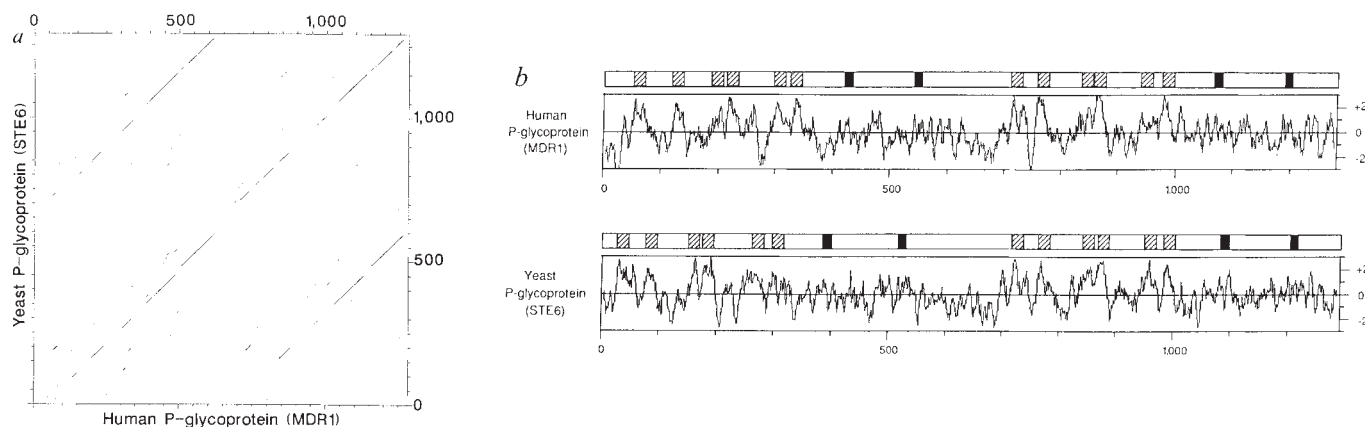


FIG. 2 Comparison of the human and yeast P-glycoproteins. a, Graphic matrix comparison of the predicted amino-acid sequences of the human *MDR1* and the yeast *STE6* proteins. The comparison plot was generated by the COM-PARE/DOTPLOT programs from the University of Wisconsin Genetics Computer Group³², using the mutational probability data matrix¹⁰ for scoring. Analysis used the window/stringency method, with a window of 30 and a stringency of 30. Numbering on each axis refers to amino-acid residues. b, Hydropathy profiles of the human and yeast P-glycoproteins. The hydropathy

plots were generated using the algorithm and hydrophobicity values of Kyte and Doolittle³³, for a window size of 9 residues; hydrophobic regions fall above the centre line and hydrophilic regions below. Schematic representations of the proteins are presented above each plot. The 12 putative transmembrane segments in each sequence are indicated by the hatched boxes. The pair of solid boxes in the hydrophilic region in each half of the P-glycoprotein molecule indicate the sequences which fit the consensus for a nucleotide binding site.

tional maturation and secretion of the **a**-factor pheromone¹⁶. The two nearly identical secreted forms of **a**-factor are processed from larger precursors; the carboxyl group of the C-terminal Cys residue of the processed precursors is methylated, and the side chain of Cys is covalently modified to yield a farnesyl thioether¹⁷. The precursors of **a**-factor contain neither a recognizable signal sequence nor potential N-linked glycosylation sites, suggesting that processing and secretion of **a**-factor does not occur by the 'classical', signal sequence-recognizing secretory pathway. Indeed, a preliminary report suggested that **a**-factor is secreted by conditional secretory (*sec*) mutants at non-permissive temperature¹⁸.

We assayed production of **a**-factor by cells carrying either a *ste6* deletion (*ste6-Δ1::HIS3*) or one of a series of *sec* mutations which, at non-permissive temperature, block protein export at different steps of the classical pathway¹⁹. As shown in the left panel of Fig. 3, no detectable **a**-factor was secreted by the *MATa ste6Δ* strain. In contrast, *sec* mutants blocked at either early (*sec53*), intermediate (*sec18*, *sec7*), or late (*sec1*) steps of the secretory pathway, secreted **a**-factor at both permissive and non-permissive temperatures. In control experiments with *MATa* cells carrying the same mutations, secretion of α -factor was unperturbed by the *ste6* deletion, whereas shift-up of the *sec* mutants to non-permissive temperature efficiently blocked α -factor secretion (Fig. 3, right panel). Thus, in contrast to α -factor, whose secretion is mediated by the classical pathway¹⁹, **a**-factor pheromone is exported through an apparently distinct and independent secretory pathway.

We propose the following scheme for the STE6 P-glycoprotein-mediated secretion of **a**-factor. The newly formed **a**-factor precursors acquire C-terminal post-translational modifications¹⁷ that render them lipophilic. We envisage that the modified precursors or their proteolytically processed products partition via their hydrophobic moieties into the lipid bilayer of the plasma membrane, where they eventually encounter the STE6-encoded P-glycoprotein which catalyses their ATP-dependent transport out of the cell. A similar process is thought to take place in multidrug-resistant mammalian cells: various hydrophobic, but otherwise apparently unrelated, cytotoxic drugs partition into the plasma membrane where the P-glycoprotein 'pump' catalyses their efflux.

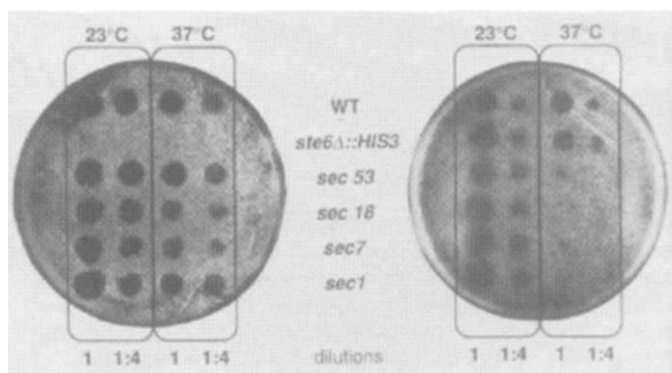
The multidrug-resistant phenotype is also known in *S. cerevisiae*²⁰. Previous work described mutants in three distinct *PDR* (pleiotropic drug resistance) genes²¹ and also in the *PMA1* gene (which encodes the plasma membrane ATPase²²), that are cross-resistant to a variety of apparently unrelated drugs. None of these genes is allelic to *STE6*. We compared wild-type *MATa* cells, congenic cells carrying the *ste6* deletion allele, and cells harbouring a high copy number 2 μ m plasmid containing the *STE6* gene, for their ability to grow in the presence of various cytotoxic compounds, which included benomyl, nocodazole, doxorubicin (Adriamycin), cycloheximide, trichodermin, chloramphenicol and oligomycin. Despite the ~30-fold overexpression of the *STE6* gene in the *STE6*-2 μ m strain (data not shown), our assays failed to detect a significant increase in resistance to any of the drugs tested. We note that overexpression of the mouse *MDR2* gene in transfected drug-sensitive mammalian cell lines also fails to confer multidrug resistance, despite an overall homology of 85% between the MDR1 and MDR2 proteins². We also note that Southern hybridization analysis of *S. cerevisiae* genomic DNA using probes from the *STE6* gene indicates that additional P-glycoprotein homologues could be present in yeast (data not shown).

Both the yeast STE6-encoded P-glycoprotein and the *E. coli* *hlyB* gene product are components of protein translocation systems distinct from the classical signal sequence-recognizing secretory pathway. By analogy, the function of at least some of the different members of the *MDR* gene family in mammalian cells may include the transmembrane transport of specific cellular proteins or peptides. One candidate for secretion by a P-glycoprotein mediated pathway is the lymphokine interleukin-1 (IL-1), whose synthesis and secretion resemble the biogenesis of **a**-factor in yeast. The IL-1 precursors lack recognizable signal sequences²³, are covalently modified by lipid (myristic acid)²⁴, and are apparently absent from the organelles of the classical secretory pathway²⁵. It is possible that, with both **a**-factor and IL-1, hydrophobic post-translational modifications serve at least in part as functional analogues of the classical signal sequence, allowing targeting of these proteins to distinct P-glycoprotein-mediated secretion pathways.

Physiological substrates of the higher eukaryotic MDR proteins and the rules of substrate recognition remain to be

FIG. 3 Secretion of **a**-factor and α -factor mating pheromones by *ste6Δ* and *sec* strains of *S. cerevisiae*. Culture supernatants from cells incubated at either permissive (23 °C) or non-permissive (37 °C) temperature were prepared and assayed for the presence of secreted **a**-factor (left panel) or α -factor (right panel) by a growth arrest bioassay. For the analysis of **a**-factor production, the strains examined were: wild-type (WT) strain JPY200 (*MATa STE6*); JPY201 (*MATa ste6-Δ1::HIS3*); VB13-5A (*MATa sec53-6*); VB10-4C (*MATa sec18*); VB8-9C (*MATa sec7*); VB12-1B (*MATa sec1-1*). Assays were carried out using the tester strain XBH8-2C, which carries the *sst2* mutation rendering it hypersensitive to growth inhibition by **a**-factor³⁴. For α -factor production, the strains assayed were: wild-type (WT) strain JPY203 (*MATa STE6*); JPY202 (*MATa ste6-Δ1::HIS3*); VB13-3B (*MATa sec53-6*); VB10-8C (*MATa sec18*); VB8-3A (*MATa sec7*); VB12-4A (*MATa sec1-1*). The tester strain used was JY383 (*MATa sst1-3*), which is hypersensitive to α -factor³⁴.

METHODS. Yeast strains were grown to mid-exponential phase in rich (YPD) medium³⁵ at 23 °C. The cultures were divided in half, the cells were pelleted by centrifugation at 5,000 *g* for 5 min at room temperature, resuspended, washed in YPD, and pelleted once again. The cells were then resuspended in fresh, prewarmed YPD at either 23 °C or 37 °C, and grown at these two temperatures for 2 h. Cell-free supernatants were prepared from the cultures by centrifugation and immediately assayed for the presence of either **a**-factor or α -factor pheromone. Approximately 5×10^5 cells of either of the two tester strains, XBH8-2C (*MATa sst2-4*) for assaying **a**-factor, or JY383 (*MATa sst1-3*) for α -factor, were spread onto a YPD plate. Samples (3 μ l) of either undiluted or four-fold diluted culture supernatants were spotted directly onto the lawns, and the plates were incubated at 23 °C. After 2–3 days of growth, the plates were examined for clear zones at the position of the sample spots, indicating the arrest of growth of the tester strain.



determined. Analysis of *STE6*, the yeast *MDR* homologue, should provide further insights into P-glycoprotein-mediated secretion. □

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- Bradley, G., Juranka, P. F. & Ling, V. *Biochim. biophys. Acta* **948**, 87–128 (1988).
- Croop, J. M. *et al. Molec. cell. Biol.* **9**, 1346–1350 (1989).
- Wilson, K. L. & Herskowitz, I. *Molec. cell. Biol.* **4**, 2420–2427 (1984).
- Gerlach, J. H. *et al. Nature* **324**, 485–489 (1986).
- Finley, D. *et al. in Ubiquitin* (ed. Rechsteiner, M.) 39–75 (Plenum, New York, 1988).
- Mackman, N. *et al. EMBO J.* **6**, 2835–2841 (1987).
- Ames, G. F.-L. *A. Rev. Biochem.* **55**, 397–425 (1986).
- Gros, P., Croop, J. & Housman, D. *Cell* **47**, 371–380 (1986).
- Chen, C. *et al. Cell* **47**, 381–389 (1986).
- Dayhoff, M. O., Barker, W. C. & Hunt, T. L. *Meth. Enzym.* **91**, 534–545 (1988).
- Eisenberg, D., Schwartz, E., Komaromy, M. & Wall, R. *J. molec. Biol.* **179**, 125–142 (1984).
- Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. *EMBO J.* **1**, 945–951 (1982).
- Finley, D., Özkaynak, E. & Varshavsky, A. *Cell* **48**, 1035–1046 (1987).
- Wilson, K. & Herskowitz, I. *Genetics* **155**, 441–449 (1987).
- Wilson, K. & Herskowitz, I. *Proc. natn. Acad. Sci. U.S.A.* **83**, 2536–2540 (1986).
- Michaelis, S. & Powers, S. in *Molecular Biology of Intracellular Protein Sorting and Organelle Assembly* (eds Bradshaw, R. A., McAlister-Henn, L. & Douglas, M. G.) 193–202 (Liss, New York, 1988).
- Anderegg, R. J., Betz, R., Carr, S. A., Crabb, J. W. & Duntze, W. *J. biol. Chem.* **263**, 18236–18240 (1988).
- Sterne, R. E. & Thorner, J. *J. Cell Biol.* **103**, 189a (1986).
- Julius, D., Schekman, R. & Thorner, J. *Cell* **36**, 309–318 (1984).
- Rank, G. H., Robertson, A. J. & Phillips, K. L. *Genetics* **80**, 483–493 (1975).
- Balzi, E., Chen, W., Ulaszewski, S., Capieaux, E. & Goffeau, A. *J. biol. Chem.* **262**, 16871–16879 (1987).
- Ulaszewski, S., Balzi, E. & Goffeau, A. *Mol. gen. Genet.* **10**, 359–364 (1986).
- March, C. J. *et al. Nature* **315**, 641–647 (1985).
- Bursten, S. L., Locksley, R. M., Ryan, J. L. & Lovett, D. H. *J. clin. Invest.* **82**, 1479–1488 (1988).
- Singer, I. I. *et al. J. exp. Med.* **167**, 389–407 (1988).
- Johnson, A. D. & Herskowitz, I. *Cell* **42**, 237–247 (1985).
- Kronstad, J. W., Holly, J. A. & MacKay, V. L. *Cell* **50**, 369–377 (1987).
- Zaret, K. S. & Sherman, F. *Cell* **28**, 563–573 (1982).
- Zalkin, H., Paluh, J. L., van Cleemput, M., Moye, W. S. & Yanofsky, C. *J. biol. Chem.* **259**, 3985–3992 (1984).
- Mortimer, R. K. & Schild, D. in *The Molecular Biology of the Yeast Saccharomyces. I. Life Cycle and Inheritance* (eds Strathern, J. N., Jones, E. W. & Broach, J. R.) 641–651 (Cold Spring Harbor Laboratory, New York, 1981).
- Özkaynak, E. & Putney, S. D. *Biotechniques* **5**, 770–773 (1987).
- Devereux, J., Haeblerli, P. & Smithies, O. *Nucleic Acids Res.* **12**, 387–395 (1984).
- Kyte, J. & Doolittle, R. F. *J. molec. Biol.* **157**, 105–132 (1982).
- Chan, R. K. & Otte, C. A. *Molec. cell. Biol.* **2**, 11–20 (1982).
- Sherman, F., Fink, G. R. & Lawrence, C. W. *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, New York, 1979).

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Substrate specificity and affinity of a protein modulated by bound water molecules

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WATER molecules influence molecular interactions in all biological systems, yet it is extremely difficult to understand their effects in precise atomic detail. Here we present evidence, based on highly refined atomic structures of the complexes of the L-arabinose-binding protein with L-arabinose, D-fucose and D-galactose, that bound water molecules, coupled with localized conformational changes, can govern substrate specificity and affinity. The atoms common to the three sugars are identically positioned in the binding site and the same nine strong hydrogen bonds are formed in all three complexes. Two hydrogen-bonded water molecules in the site contribute further to tight binding of L-arabinose but create an unfavourable interaction with the methyl group of D-fucose. Equally tight binding of D-galactose is attained by the replacement of one of the hydrogen-bonded water molecules by its —CH₂OH group, coordinated with localized structural changes which include

a shift and redirection of the hydrogen-bonding interactions of the other water molecule. These observations illustrate how ordered water molecules can contribute directly to the properties of proteins by influencing their interaction with ligands.

L-Arabinose-binding protein (ABP) serves as an initial receptor for the high-affinity active transport or permease system in Gram-negative bacteria. Whereas L-arabinose (Ara) and D-galactose (Gal) are both excellent substrates of ABP (K_d values of 0.98×10^{-7} M and 2.3×10^{-7} M, respectively), D-fucose (Fuc) is bound less tightly ($K_d = 3.8 \times 10^{-6}$ M)¹. All three monopyranosides bind to ABP with comparably fast association rate constants ($0.8\text{--}2.4 \times 10^7$ M⁻¹ s⁻¹), and the ratio of the dissociation rate to the association rate of each sugar agrees well with K_d obtained by equilibrium binding measurements¹. Thus, substitution of the equatorial or (R) proton at position C5 of the L-arabinose, in the ⁴C₁ conformation, by the bulky —CH₂OH group does not essentially change the tightness or the kinetics of ligand binding, but substitution by the smaller, apolar —CH₃ does reduce the binding affinity by ~40-fold. Also, the thermodynamic parameters for binding of Ara and Gal are basically identical^{2,3}.

To investigate the unusual substrate specificities of ABP, we undertook a crystallographic structure refinement to obtain accurate atomic structures of the complexes of ABP with Ara, Fuc and Gal. The highly refined 1.7-Å crystal structure of the ABP-Ara complex (R -factor = 0.137; ref. 4) (Fig. 1) has facilitated a thorough refinement of the ABP-Gal complex at 1.8-Å resolution (R -factor = 0.132) and of ABP-Fuc at 1.9-Å resolution (R -factor = 0.134). As observed in the ABP-Ara structure⁴, the sugar-filled binding sites in the two other complexes also show extremely well-defined electron density, with essentially all the atoms exhibiting thermal parameters twofold lower than the average for the entire structures. Figures 2 and 3 further attest to the high quality and therefore the accuracy of the refined structures.

The ABP-Fuc and ABP-Gal complexes share several prominent features originally found in the ABP-Ara complex (Fig. 1)^{4–6}. Each monopyranoside fills and is completely enclosed by the cleft between the two globular domains of ABP. The pyranose rings and the atoms common to all three monopyranosides are identically positioned within the sugar-binding site. The sugars are held in place by strong hydrogen bonds with mainly planar, polar side-chains and by van der Waal's contacts. The hydrogen bonds take on three forms: cooperative hydrogen bonds, of the simple form (NH)_n → OH → O, where OH is a sugar hydroxyl, NH is the hydrogen bond donor, O is the acceptor and $n = 1$ or 2; bidentate hydrogen bonds, such as those involving Asn 232 and Arg 151; and, networked hydrogen bonds, such as those shown in Fig. 1b. The hydrophobic patch, composed of C3-H, C4-H and C5-H, on the β-face of the pyranose ring, is partially stacked with the β-face of the indole ring of Trp 16, and the exposed C2-H on the α-face is close to the ε-methyl group of Met 204 (Fig. 1). The substrate-binding site is able, by a simple mechanism, to recognize either the α- or the β-anomeric form of the pyranosides. Further details of the nature and importance of hydrogen bonds, van der Waal's contacts, apolar stacking interactions and other features of the ABP-Ara complex, which are also applicable to the other two complexes, have been described elsewhere^{4–6}.

With the exception of the hydrogen bond between water molecule 310 and the sugar-ring oxygen, which is found in both ABP-Ara and ABP-Fuc complexes but not in ABP-Gal (Figs 2–4) identical hydrogen bonds are associated with OH1, OH2, OH3, OH4 and O5 of the monopyranosides in all three complexes (see Fig. 1). Relative to Ara binding, the —CH₃ group of Fuc causes a rotation of Met 108, a movement of its flanking residues and a very slight shift in the position of water 311 (Figs 2 and 4). The rotation puts the Met methyl group within 3.7-Å of the methyl group of Fuc, creating a favourable non-polar interaction similar to the one involving Met 204 (Fig. 1a). Had

FIG. 1 Atomic interactions between ABP and α - or β -L-arabinose as obtained from the refined 1.7-Å resolution structure of the ABP-Ara complex⁴⁻⁶. *a*, Stereoscopic view of the hydrogen-bonding and apolar-stacking interactions in the complex. Carbon atoms are depicted as single circle, oxygen atoms as double circles and nitrogen atoms as triple circles. All the hydroxyls and the ring oxygen participate in the formation of 10 hydrogen bonds (broken lines) with overall mean distance of 2.81 (± 0.15) Å and mean angle of 164 (± 9)° (ref. 4). Water molecules 309 and 310 are roughly tetrahedrally coordinated, whereas water 311 is roughly trigonally coordinated. Water 310 is hydrogen bonded to O5 of the sugar ring (distance = 2.77 Å), OG1 of Thr 147 (2.78 Å), peptide O of Asp 89 (2.71 Å) and water 311 (2.84 Å). Water 311 forms further hydrogen bonds with Asp 89 OD2 (2.72 Å) and Met 108 peptide NH (2.86 Å). *b*, Schematic diagram of the hydrogen bonds in the ABP-Ara complex, also showing networks of hydrogen bonds.

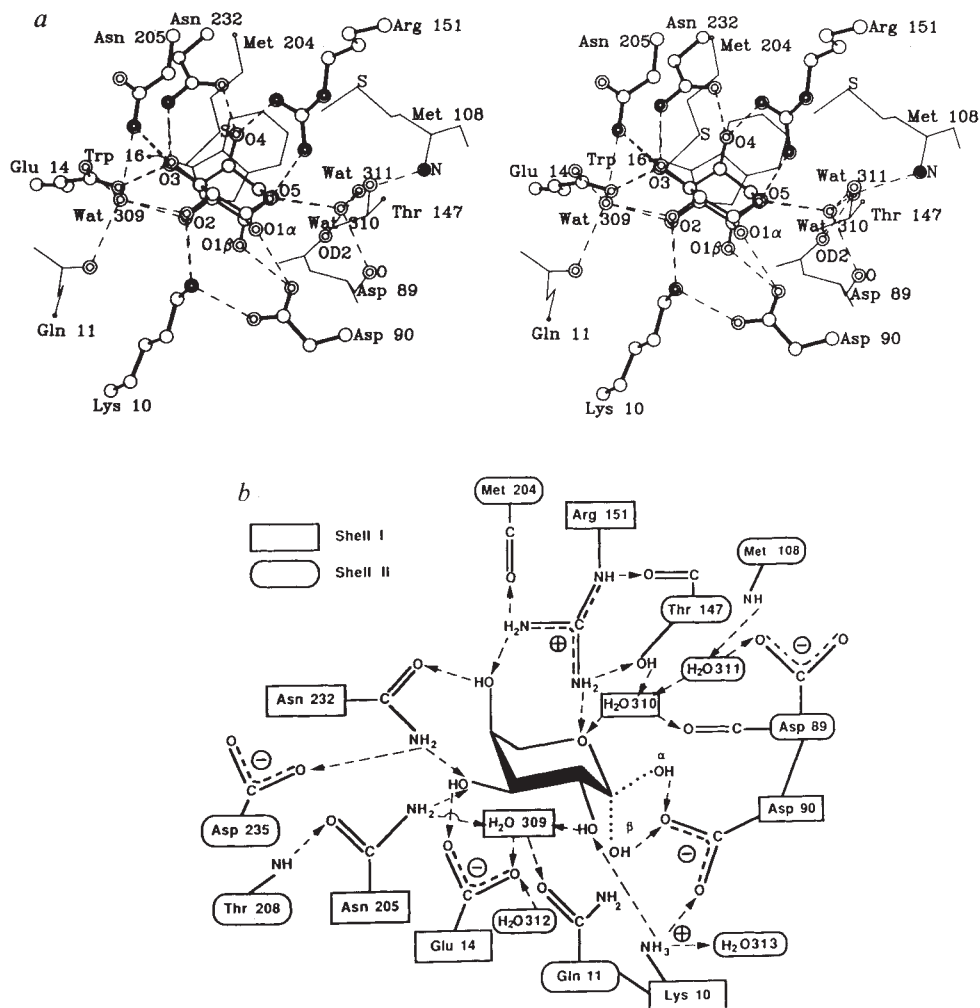
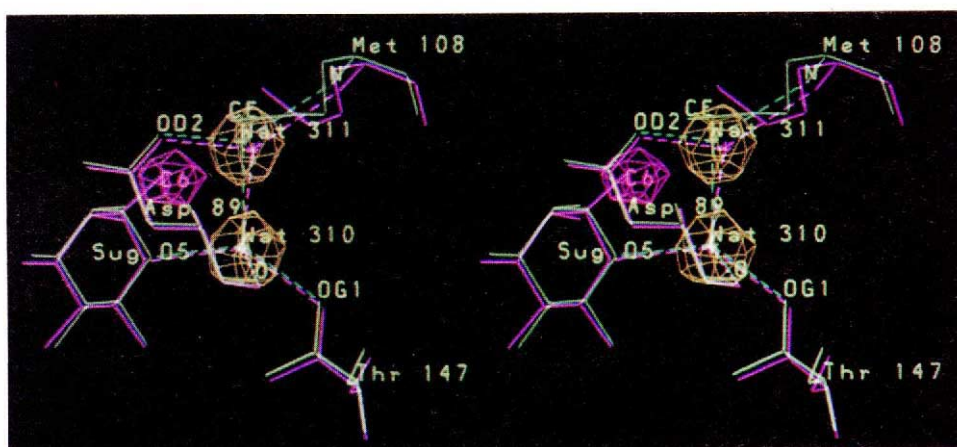
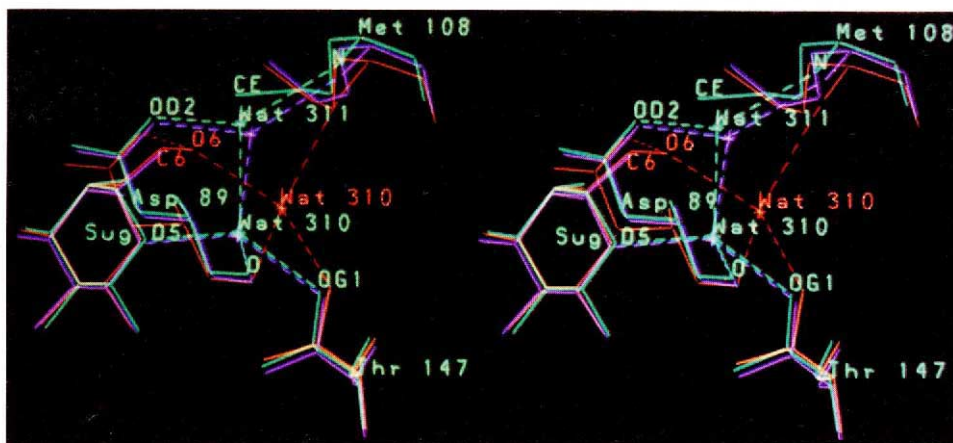


FIG. 2 Stereoscopic view of the refined ABP-Fuc (magenta) structure superimposed on ABP-Ara complex (green). Both Fuc anomers are bound with hydrogen-bonds identical to those in the ABP-Ara complex (see Fig. 1*a*), but for clarity only the refined sugar models with equatorial anomeric hydroxyl (α -L-Ara and β -D-Fuc) are shown. Only the hydrogen bonds (broken lines) associated with water 310 and water 311 are shown. The electron density maps were obtained with $(|F_o| - |F_c|, \alpha_c)$ residual maps in which the contribution of the methyl group of Fuc in the ABP-Fuc and water 310 and water 311 in the ABP-Ara are omitted in the structure factor calculations (the density was contoured at 6σ of the difference map). Except for those shown, no other differences in electron density were observed. The ABP-Fuc and ABP-Gal complexes were each prepared by exhaustively dialysing purified native ABP against a solution containing the appropriate sugar. The stoichiometry was shown to be one molecule of sugar bound per ABP molecule by using radioactively labelled sugars. This stoichiometry was confirmed by the structure refinement. Single crystals of each of the complexes were obtained using the same conditions as for the ABP-Ara crystals¹⁰. As crystals of both complexes are isomorphous with the ABP-Ara complex crystals^{4,10}, restrained least squares refinement¹¹ of the structures of both complexes was straightforward. The refinement strategy is similar to the one used for



the ABP-Ara structure⁴. In the final round of refinements, no restraints were applied to the non-bonded contacts, including hydrogen-bond distances, between the sugars and ABP. Water molecules were modelled as oxygen atoms. Refinement of the 2,529 atomic coordinates (including water molecules) of the ABP-Fuc structure proceeded to an *R*-factor of 0.134 for 18,780 reflections in the shell from 10–1.9 Å. The r.m.s. deviations from accepted protein stereochemistry are: 0.03 Å for bond distances; 0.06 Å for bond-angle distances; 0.011 Å for deviations from planarity of planar groups; 2.3° for deviation from 180° for the ω -angle of the peptide groups and 0.09 Å³ for the chiral volume.

FIG. 3 Stereo view of the refined structure of the ABP-Gal complex (red) in the immediate vicinity of the $-\text{CH}_2\text{OH}$ of Gal superimposed on the refined ABP-Ara complex (green). The hydrogen-bond parameters in the ABP-Gal complex are similar to the ABP-Ara complex. For clarity only the refined model of the β -D-Gal is shown. The difference electron density (contoured at 6σ of the difference map) of each of the complexes was determined as described in Fig. 2. The difference density for the ABP-Ara is identical to that shown in Fig. 2. C6-O6 of Gal and water 310 were not included in the calculation of the difference density of the ABP-Gal complex. Water 310 hydrogen-bonds with peptide O of Asp 89 (distance = 2.78 Å), γ -OH of Thr 147 (2.85 Å), Gal O6 (2.76 Å) and peptide NH of Met 108 (3.34 Å) (Fig. 3). The OH6 group of Gal further donates a hydrogen bond to Asp 89 OD2 (2.75 Å). The χ^5 or C4-C5-C6-O6 torsion of the bound D-galactose is 190° , hence hydrogen bonding between OH6 and the O5 ring oxygen is not possible. The ABP-Gal complex was prepared and crystallized as briefly described for ABP-Fuc in Fig. 2 legend. Refinement of the 2,576 atomic coordinates (including water molecules) of the ABP-Gal structure proceeded to an *R*-factor of 0.132 for 18,860 reflections in the shell from 10–1.8 Å. The



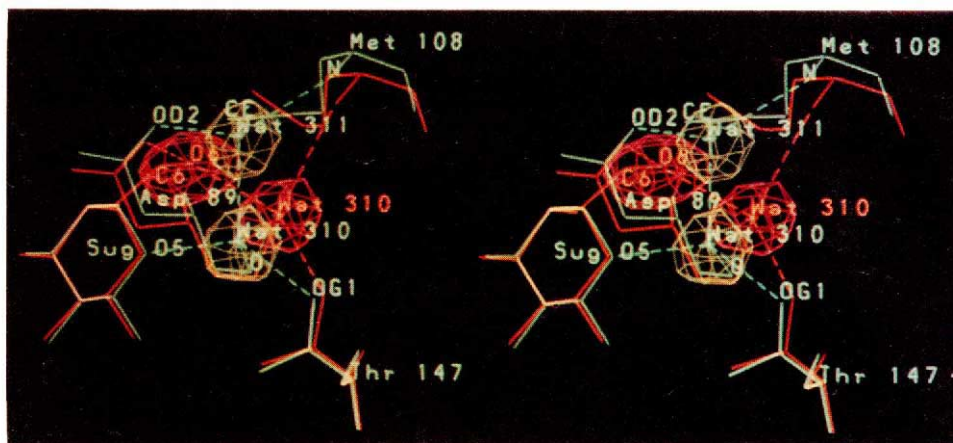
r.m.s. deviations from accepted protein stereochemistry are: 0.03 Å for bond distances, 0.05 Å for bond angle distances; 0.02 Å for deviations from planarity of planar groups; 4° for deviation from 180° for the ω -angle of the peptide groups; and 0.25 Å³ for the chiral volume. Note that the sizes of the electron density (contoured at the same level) in Figs 2 and 3 clearly reflect, in increasing order, the atomic number of the methyl group (modelled as C atom) of Fuc, individual water molecule (modelled as O atom) and C6-O6 group of Gal.

there been no change from the position in the ABP-Ara, the two methyl groups would be too close by ~ 2.6 -Å. However, the apolar methyl group of Fuc is within 3.2-Å of water 310, 3.4-Å of water 311 and 3.8-Å of Asp 89 oxygen atom OD2.

The $-\text{CH}_2\text{OH}$ group of the D-galactose induces many more local changes, especially in the structure of hydrogen-bonded water molecules 310 and 311. As shown in Fig. 1, in the ABP-Ara complex, water 310 is hydrogen bonded to O5 of the sugar ring, OG1 oxygen atom of Thr 147, peptide O of Asp 89 and water 311. Water 311 is further hydrogen-bonded to Asp 89 OD2 and Met 108 peptide NH. The following highly coordinated changes distinguish ABP-Gal from ABP-Ara (Figs 3 and 4) and largely account for the novel stereospecificity of ABP. Water molecule 311 is dislodged by the close approach of the hydroxymethyl group; consequently, three hydrogen bonds, with the acceptor oxygen atom OD2 of Asp 89 and the donors water 310 and peptide NH of Met 108, are lost. Concomitantly, water 310 is shifted and no longer able to donate a hydrogen bond to the

sugar-ring oxygen. By shifting along an arc, however, water 310 retains the hydrogen bonds with peptide O of Asp 89 and γ -OH of Thr 147 and redirects the other two, from Ara O5 to Gal O6 and from water 311 to peptide NH of Met 108 (Fig. 3). Thus, water 310 is still approximately tetrahedrally coordinated. The OH6 group of Gal further donates a hydrogen bond to Asp 89 OD2. Finally, three side-chain conformations are changed to achieve favourable interactions (Fig. 3); Asp 89, to form hydrogen bonds with Gal OH6 and water 310; Thr 147, to maintain tetrahedral coordination of water 310; Met 108, similarly to ABP-Fuc complex (Fig. 2), to create a favourable non-polar interaction between its methyl group and the C6-methylene of Gal (3.7-Å) and to form a hydrogen bond with water 310. Remarkably, despite the considerable difference in local conformation between ABP-Ara and ABP-Gal, there is only one more hydrogen bond to Gal than to Ara, and the net effect of the conformational changes is one less hydrogen bond in ABP-Gal than in the ABP-Ara complex. It is also important

FIG. 4 Stereo view of the superimposed refined models of ABP-Ara (green), ABP-Fuc (magenta), and ABP-Gal (red) complexes. Clearly shown are the coordinated movements of residues in the ABP-Fuc and ABP-Gal complexes relative to the ABP-Ara complex. Although the carboxylate side-chain of Asp 89 in the ABP-Fuc and ABP-Ara is superimposable, it undergoes a torsional rotation in the ABP-Gal complex in order to form an H-bond with OH6 of Gal. There is a similar rotation of the methyl group of Met 108 in ABP-Fuc and ABP-Gal, which is due to steric hindrance with the C6 sugar atom. Other hydrogen-bonding interactions common to the three complexes are virtually identical or unperturbed, consistent with the pyranose ring of all three sugars occupying the same position in the binding site.



to the stability of the ABP-Gal complex that these changes result in the pairing of all hydrogen-bonding groups.

These results demonstrate several roles for water molecules in substrate binding. Water molecules contribute to the stability of a particular complex by mediating hydrogen bonds between the functional groups of the protein and the sugar and by filling in potential voids or holes inside the enclosed binding site. The interactions of the water molecules, albeit in some instances attained by conformational rearrangements, ensure that sequestered polar groups, especially those with active or donatable protons, are paired; an active proton unable to make a hydrogen bond could destabilize the system by as much as 7 kcal mol⁻¹ (ref. 7). Water molecules occupy less space than the usual large, polar, planar residues used in sugar binding, but still engage in as many as four hydrogen bonds. The strategic location, the mobility, the polarity and the hydrogen-bonding interchangeability and capacity of the paired water molecules are major factors in enabling the binding site of ABP also to easily accommodate D-galactose. Moreover, the concomitant release of the ordered water 311 molecule, presumably to the bulk solvent, with the formation of the ABP-Gal complex contributes favourably to entropy.

One notable feature of the comparatively high affinities of the three complexes is the large number of hydrogen bonds and van der Waal's contacts (~50) (refs 4 and 6), which results in a packing interface between functional groups (from residues and water molecules) and the buried sugar which is highly efficient^{5,8} or as good as that observed inside proteins⁹. Although the complementarity between types of surfaces is good, particularly in polar areas, across this interface in both ABP-Ara

and ABP-Gal complexes (Figs 1, 3 and 4), there is a defect in the ABP-Fuc complex. Indeed, we attribute the weaker binding of D-fucose primarily to the proximity and non-complementarity of its methyl group and water molecules 310 and 311 and Asp 89 (Figs 2 and 4).

Our results show that ordered water molecules are important in binding L-arabinose and D-galactose, which are neither epimers nor distereoisomers, and that aspartic residues are essential for binding of sugar anomers to the L-arabinose- and D-galactose-binding proteins^{4,5} (Fig. 1) and of the 4-epimers D-galactose and D-glucose to the D-galactose-binding protein⁵. It is noteworthy that the D-galactose-binding protein binds neither L-arabinose nor D-fucose, simply because the protein lacks the equivalent of the hydrogen-bonded water molecules found in ABP (ref. 5; N.K.V. and F.A.Q., unpublished data). Finally, the nature of the binding site of ABP suggests that there is scope for designing analogues or inhibitors with functional groups capable of displacing bound water molecules and directly hydrogen-bonding with the protein. □

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1. Miller, D. M., Olson, J. S., Pflugrath, J. W. & Quiocho, F. A. *J. biol. Chem.* **258**, 13665-13672 (1983).
2. Clark, A. F., Gerken, T. A. & Hogg, R. W. *Biochemistry* **21**, 2227-2233 (1982).
3. Fukada, H., Sturtevant, J. M. & Quiocho, F. A. *J. biol. Chem.* **258**, 13193-13198 (1983).
4. Quiocho, F. A. & Vyas, N. K. *Nature* **310**, 381-386 (1984).
5. Vyas, N. K., Vyas, M. N. & Quiocho, F. A. *Science* **242**, 1290-1295 (1988).
6. Quiocho, F. A. *Curr. Topics Micro. Immun.* **139**, 136-148 (1988).
7. Jacobson, B. L. & Quiocho, F. A. *J. molec. Biol.* **204**, 783-787 (1988).
8. Quiocho, F. A. *Pure appl. Chem.* **61**, 1293-1306 (1989).
9. Richards, F. M. *Rev. biophys. Bioeng.* **6**, 151-176 (1977).
10. Quiocho, F. A., Philips, G. N. Jr, Parsons, R. G. & Hogg, R. W. *J. molec. Biol.* **86**, 491-493 (1974).
11. Hendrickson, W. A. *Meth. Enzym.* **115**, 252-270 (1985).

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Focus on summer products

This summer season provides a selection of new products: a rabbit model for AIDS, a monoclonal antibody specific for the retinoblastoma gene product, and a reagent for keeping cell lines mycoplasma-free.

APPLIED Biosystems has designed a **DNA synthesizer** specifically for producing oligonucleotide primers used in the PCR process (*Reader Service No. 100*). The \$12,000 (US) PCR-MATE has fast cycle times that permit the synthesis of two primers in less than four hours, says the company. The system produces high-quality DNA, eliminating the problems associated with base modifications in PCR primers, which could lead to misincorporation or nonspecific priming during amplifi-



Applied Biosystems' "oligo" synthesizer.

cation. Applied Biosystems says the instrument's base-coupling efficiency gives reliable results with PCR-length primers without the need for time-consuming HPLC or gel purification. The DNA synthesizer has a compact benchtop design and can accommodate different scales of synthesis to suit user needs. PCR-MATE can be used to complement the already available Perkin-Elmer Cetus' DNA Thermal Cycler and GeneAmp DNA amplification reagents.

Valuable cell lines can be kept mycoplasma-free using Flow's new **mycoplasma-removal agent**, which was produced in collaboration with the Japanese Dianippon Pharmaceutical Company Ltd (*Reader Service No. 101*). The MRA reagent — a water-soluble oxo-quinoline carboxylic acid derivative — is supplied as a ready-to-use solution. Flow says MRA is one-thousand times more effective at removing even low concentrations of mycoplasma than other agents; it may also be used as a preventative measure where there is a likelihood of contamination. The removal agent takes effect within a week and has minimal non-target cell activity, says the company. It comes in a 5-ml vial and the 50 mg ml⁻¹ solution will decontaminate up to 25 cultures.

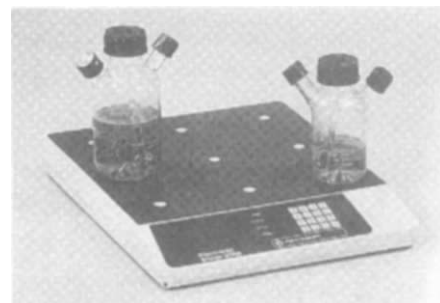
As of November 1989, the research community will have access to the patented **rabbit animal model** for use in AIDS testing, commercially produced by RRI in conjunction with the National Institute of

Mental Health (*Reader Service No. 102*). The protocol for infecting New Zealand White rabbits with human immunodeficiency virus-1 (HIV-1) was developed by the National Institutes of Health in 1988, and is expected to be used extensively in the testing and evaluation of AIDS-related vaccines, drugs and other pharmaceutical agents. Using double-blind protocols, RRI will administer test substances to quarantined rabbits, and carry out a series of analyses, including serum testing for antibodies to HIV-1. Clients can make changes to the standard protocol and can request additional services, such as necropsy. Client confidentiality and the security of all test materials submitted is guaranteed, says RRI. Prices for RRI's services are dependent upon the numbers of rabbits tested, the complexity of the protocol and the duration of the study.

Monoclonal antibodies specific for the human **retinoblastoma gene product** are now available commercially from PharMingen (*Reader Service No. 103*). The retinoblastoma gene encodes a protein that is thought to be important in growth control: it is bound and probably functionally inactivated by viral transforming proteins (*Nature* **334**, 124-129; 1988) and is absent in many tumours. PharMingen says its PMG3-245 MAb recognizes variously phosphorylated forms of the retinoblastoma protein. The antibody can be used in Western blots, to immunoprecipitate retinoblastoma proteins, or in indirect immunofluorescence staining to study the role the retinoblastoma gene product plays in cell cycling and differentiation. A 0.1-mg vial of the MAb costs \$150 (US). PharMingen also sells purified isotype controls, and enzyme-conjugated and unconjugated purified rabbit anti-mouse antibodies, as second-step reagents.

Benchtop accessories

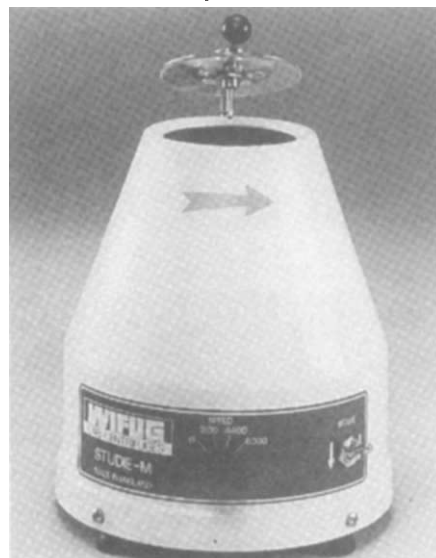
Fisher Scientific has a new line of **electronic stirrers** with no moving parts, motors or magnets (*Reader Service No. 104*). The Series 2000 microprocessor-controlled stirrers use continuous-coil electronic field technology and operate at speeds of 1-1,500 r.p.m. — giving smooth rotation even at low r.p.m., says the company. The unit can stir in both forward and reverse directions, with the option of a shaking action. Preprogrammed rates of acceleration/deceleration minimize cell trauma and increase yields, says Fisher. The stirring time can be set to run con-



The 9-position electronic stirrer from Fisher Scientific.

tinuously or with "on/off" cycles. The stirrers can operate at temperatures between +45 °C and -5 °C. The \$1,280 (US) nine-station stirrer with separate power supply can stir nine vessels simultaneously or a rack of test tubes. To suit user and laboratory needs, there is also the \$821 (US) one-station stirrer with integral power supply. This master control unit can be used alone or to control up to eight further satellite stations, up to a distance of 100 feet away, including the \$149 (US) one-station stirrer or the \$295 (US) three-station stirrer.

For those looking for complete flexibility in the laboratory, Eltex of Sweden has the compact and portable **STUDIE M benchtop centrifuge**, which can run off a 12-V d.c. battery as well as mains (*Reader Service No. 105*). The £370 (UK) centrifuge can hold six metal cups, with rubber inserts, for 15-ml tubes. The three-step speed regulator can operate at 1,800, 4,400 and 6,000 r.p.m. A reverse-current



Battery-operated benchtop centrifuge from Eltex.

breaking system slows down the angle rotor in seconds, says the company. A transparent lid and safety lock are standard features.

To keep **pH electrodes** in sparkling condition, Radiometer Analytical A/S has a complete maintenance kit for use with all types of combined pH electrodes, glass pH electrodes and reference electrodes that use potassium chloride as a salt bridge (*Reader Service No. 106*). The GK ANNEX kit includes about three months' supply of both gentle and extra strong cleaning solutions for the removal of fat and protein deposits, 12 months' supply of potassium chloride refilling solution and crystals, and an assortment of utensils. A



Radiometer Analytical's pH maintenance kit keeps pH electrodes ship-shape.

logbook is included that contains advice on electrode handling and maintenance, and provides space for recording details of the electrode's history and dates of calibration.

A **benchtop anaerobic workstation** for use in many laboratory situations is available from Don Whitley Scientific Ltd (*Reader Service No. 107*). The £6,500 (UK) COMPACT workstation is designed to give clear visibility of experimental procedures and bare-hands access for the handling and inoculation of petri dishes.



Anaerobic workstations from Don Whitley.

Temperature, humidity and anaerobic conditions are regulated by the unit, which can accommodate up to 180 petri dishes. The capacity of the standard model can be increased to suit laboratories with larger-scale requirements.

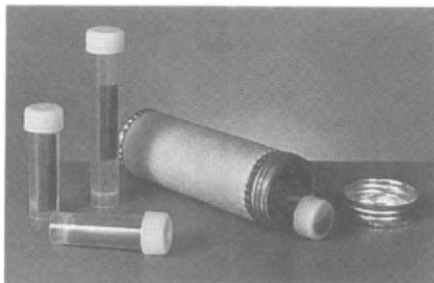
Safety first

Whatman has a **flameless heat source** that provides a safe alternative to bunsen burners for sterilizing instruments and heating flasks (*Reader Service No. 108*). The Safe Air Heater is an electric hot-air blower that produces a stream of hot air. Whatman says the Safe Air blower



Electric hot-air blower from Whatman. reaches a maximum nozzle temperature of 500 °C within two minutes.

With the current concern over the safe transport and storage of etiological agents, Elkay Products, Inc. announces a new line of 5- and 10-ml **transport tubes** (*Reader Service No. 109*). Elkay says the tubes — made of precision-moulded polypropylene — are virtually shatterproof, resistant to chemicals and autoclavable.



Microbe mailers from Elkay Products, Inc.

The tubes are conical in shape and come with a free-standing base. The polyethylene screwcaps provide a uniform fit and have a leakproof seal, says Elkay. The bold graduation lines and numerals on the tubes make it easy to estimate volume, and the specially formulated ink resists scuffing and most laboratory solvents, says the company. A large area is provided for labelling, which simplifies sample identification. The 5- and 10-ml tubes are \$105 (US) and \$115 (US), respectively for 1,000, and can be supplied either sterile or non-sterile.

Those concerned with safety in the laboratory may be interested in Kewaunee Scientific Corporation's Visionaire **fume hood** (*Reader Service No. 110*). The Visionaire is designed to reduce air turbulence within the hood to contain and exhaust noxious odours and toxic fumes. An angled sash and transparent vision panel give unrestricted viewing of the hood interior while improving bypass airflow, says Kewaunee. The unit, which is

constructed on a free-standing base, has thin side panels to maximize the work-surface area. Handles and outlet nozzles for air, gas, water and vacuum lines are colour-coded for ease of identification. Interior liners that become stained or

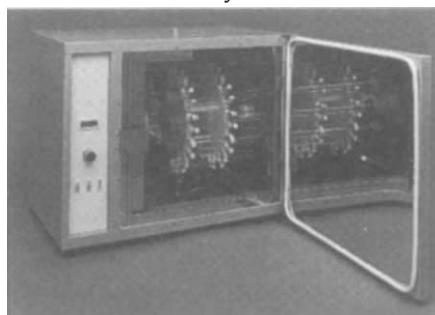


The Visionaire fume hood from Kewaunee Scientific Corporation.

damaged can be renewed individually without the need for hood replacement. Kewaunee also offers a complete laboratory design service.

High-tech hybridization

The new rotisserie-action **hybridization oven** from Hybaid Limited can be used to process up to 12 blotting membranes from Northern, Southern or Western blots, in the presence of radioactive and non-radioactive probes (*Reader Service No. 111*). The £1,695 (UK) Hybaid Hybridization Oven rotates tubes at a constant 5 r.p.m.; this ensures that the prehybridization, hybridization and washing solutions are distributed evenly over the membrane

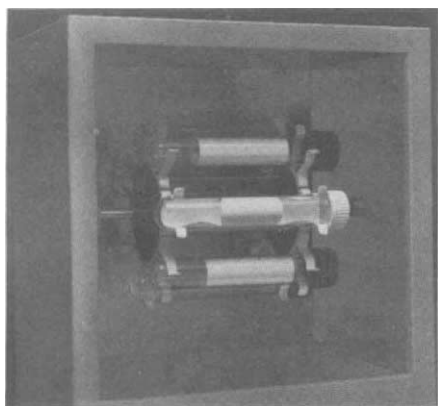


Hybaid's hybridization oven handles up to 12 membranes at once.

and reduces the volume of probe solution required — typically 10 ml for a 20 × 20-cm blot, says the company. The rotisserie unit comes in a fan-driven oven that is accurate up to 90 °C. The oven can also be used as an incubator.

Robbins Scientific Corporation offers a **hybridization incubator** that allows researchers to dispense with the water

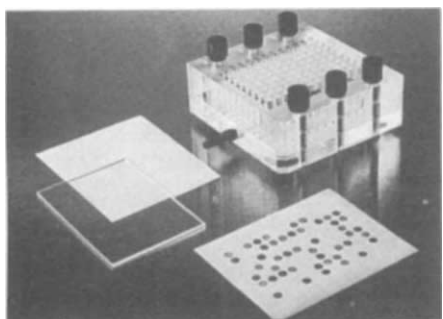
baths, plastic bags, heat sealers and needles used in the conventional processing of Northern and Southern blots (*Reader Service No. 112*). The Hybridization Incubator rotator can hold up to ten 75- to 300-mm screwcap tubes, and turns at a constant speed of 6 r.p.m. The rotational action provides more efficient wetting of the membrane and so reduces the



Rotisserie-action hybridization incubator from Robbins Scientific.

volume of probe solution, says the company. The unit can process membranes that are 1–10 inches wide and up to 20 inches in length. The temperature in the benchtop-sized incubator is electronically controlled from ambient to 90 °C, and accurate to ± 0.5 °C, says Robbins Scientific. The \$2,475 (US) incubator comes with four screwtop glass tubes.

Oncor, Inc. offers an integrated **non-radioactive hybridization and detection system** for DNA dot blots (*Reader Service No. 113*). Oncor says its system provides a quick and easy method for preparing nucleic acid targets on a membrane. The dot blot apparatus is made up of a three-tiered unit: the top plate has 96 sample loading wells, each holding a volume of 460 μ l, the middle plate supports the membrane, and the bottom plate contains a vacuum plenum with a hose-fitting port. The unit requires a vacuum source of 2–3 in. mercury to draw the fluid through the membrane. The plate interfaces are designed to prevent overtightening and cross contamination, says the company. The \$315 (US) system comes complete with dot blot apparatus, precut nylon



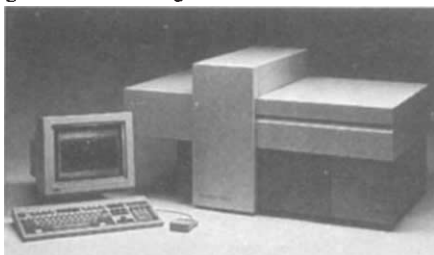
Oncor's non-radioactive dot blot system.

membrane and membrane support pad, enzymes, washing solutions, dyes, and all the blocking reagents and hybridization reagents used in the non-isotopic analysis.

Autorad analysis

Saxon Micro announces "Autograph", a new process for the **digital imaging** and evaluation of radioisotope-labelled electrophoresis gels or blots (*Reader Service No. 114*). Saxon Micro says the system's digital collimator — developed by Oxford Positron Ltd — forms a digital autoradiograph image fifty times faster than film, reducing the detection and evaluation time of most beta and gamma emitters to minutes, rather than days or weeks. The £20,000 (UK) system has a 1-mm resolution over an active image window of 220 mm $\times$ 220 mm, accommodating most gels, blots or tissue sections. The PC-based imaging software is operated using pull-down menus. Further applications software for the quantification of complex images is also available.

Film-free autoradiography is now possible, with the model 400A PhosphorImager from Molecular Dynamics (*Reader Service No. 115*). The instrument is based on a phosphor screen that absorbs and stores beta and gamma radiation emitted by isotopes used to label electrophoresis gels and blotting membranes. When the



The 400A PhosphorImager from Molecular Dynamics.

screen is scanned by the instrument's HeNe laser beam, the stored energy is released as blue photons, which are collected, digitized and stored in computer memory. The screen can then be erased for reuse. Molecular Dynamics says the phosphor technology is 100 times more sensitive than film to 32 P, and has a wider dynamic range: bands which differ by a factor of 10,000 or more can be analysed at once, eliminating the need for multiple exposures. The \$75,000 (US) PhosphorImager comes with applications software for analysing the data it generates.

Biological Vision, Inc. has developed an **image analysis system** for autoradiographs that it says can quantify and compare bands undetectable by eye, in a fraction of the time taken by conventional means (*Reader Service No. 116*). The BVI 4000 Image Analysis System's CCD camera can capture autoradiographic, fluorescent or stained data in less than a second, says

the company. Background is automatically eliminated before the system provides an illustration of the signal differences between two corresponding images. The system distinguishes between true differences and those resulting from positional distortion. Using mouse-driven cursors, differences that are of interest to the user can be highlighted and quantitated by the selective use of colour. BVI supplies application software packages for DNA and protein research, including PCR, microtitre assays, and 1- and 2-D electrophoresis. The system can also be used for most types of blots.

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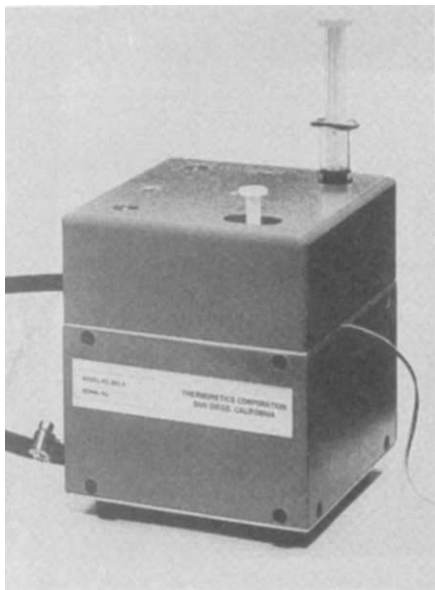
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Reader Service No. 15

Cold fusion, anyone?

For those engaged in attempts to replicate the results of Pons and Fleischmann, Thermochemicals sells a range of solid-state **calorimeters** (*Reader Service No. 117*). Available in models with capacities ranging from 1 cubic inch to 24 cubic feet, Thermochemicals says its model SEC calorimeters can measure the smallest increases in heat over time, with traceable accuracy. Sensitivity is limited only by the noise of the system under study, says the company. The calorimeters generate a proportional



Thermochemicals' SEC calorimeter for cold fusion. e.m.f. compatible with conventional recorders and computers.

Hot off the production line is Hart Scientific's **custom-designed calorimeter** for measuring, recording and analysing the heat produced by electrochemical reactions, such as that claimed in the reactions that have been dubbed "cold fusion" (*Reader Service No. 118*). The \$8,500 (US) model F400 calorimeter fits easily on the benchtop, and its reactor cavity accepts reaction vessels up to 2.5 in. in diameter and 5.5 in. deep. Hart Scientific says the model F400 measures produced heat to within 0.1 per cent. For other applications, the company also offers calorimeters with larger reactor cavities, greater sensitivity, expanded temperature ranges and computer automation.

J.M. Ney Company sells 99.9 per cent pure **palladium cathodes** for use in reproducing the cold fusion phenomenon (*Reader Service No. 119*). The company offers the alloy in shapes developed from specifications published by Pons and Fleischmann. Rods in lengths of 0.1, 0.2 and 0.4 cm, and 0.2-cm plates can be shipped to customers through overnight mail within two days, says J.M. Ney. Cathodes may also be custom-ordered.



High-purity palladium electrodes from the J.M. Ney Company.

HIV research

A **quantitative HIV-1 antibody assay** kit using colourimetric analysis has been developed by Beckman Instruments (*Reader Service No. 120*). Beckman says the ASQ HIV Kit gives non-subjective results within four hours and can analyse antibodies to core, envelope and polymerase gene products, using six recombinant peptides — p24, kp55, kp41, kp120N, kp120CC and kp66/31. The absorbance values are related to antibody reactivity and relative concentration. The test can be performed manually or automatically, using a system like the Beckman BIOMEK laboratory workstation. Beckman says the test removes the guesswork associated with conventional Western blotting methods, and provides researchers with a means of following the effectiveness of drug therapies.

Cellular Products, Inc. has teamed up with Cetus Corporation to develop a recombinant growth factor for cells of the **macrophage/monocyte lineage** (*Reader Service No. 121*). Macrophage Colony Stimulating Factor (M-CSF) — a protein that mediates cell growth, differentiation and activation — could have important applications in AIDS research, says the company. Studies have shown M-CSF to be essential for HIV replication in normal human monocytes. Cellular Products says M-CSF has other applications in studies to determine the mechanism of the M-CSF/receptor interaction, as well as in *in vitro* studies of myeloid leukaemias, trophoblast cells and choriocarcinomas — all of which express receptors for M-CSF. Tissue-grade quality M-CSF is formulated in human serum albumin and supplied in packages containing 10,000 and 100,000 units for \$100 (US) and \$500 (US), respectively.

Current catalogues

Choosing which **competent cells** to use for performing transformations need not be a problem with Bethesda Research Laboratories' updated *Guide to Frozen Competent Cells* (*Reader Service No. 122*). The guide details nine available varieties of high-performance frozen competent cells, including the new premium-performance category of MAX-Efficiency Competent

Cells, which BRL says yields the highest transformation efficiency available. The guide also contains practical information about choosing the right efficiency and host for a particular application, performing transformations, and advice on how to troubleshoot problems with transformation protocols.

Bio-Rad Laboratories announces the availability of its free, 29-page colour catalogue of new **molecular biology products** (*Reader Service No. 123*). The catalogue contains information about new products for DNA sequencing, including an RNase-based dideoxy sequencing kit and a sequencing electrophoresis cell that can accommodate six different gel sizes. Other sections include information on new kits and membranes for nucleic acid blotting and hybridization, non-isotopic screening systems, and a pulsed-field electrophoresis system, which BioRad says gives high resolution and straight lanes.

The 1989 Sigma Chemical Company **peptides catalogue** is now available (*Reader Service No. 124*). The catalogue contains a full listing of the company's bioactive peptides, small peptides and substrates, and polyamino acids. More than 600 highly characterized, high-purity synthetic bioactive peptides are included, as well as over 500 small peptides and substrates, and 150 polyamino acids. Sequence data and technical references are given for many of the products.

A catalogue of **laboratory research products** of interest to life sciences researchers is now available from Research Products International Corporation (*Reader Service No. 125*). The catalogue features radiochemicals, chemicals and vials used in liquid scintillation, instruments for monitoring radioactivity, and radiation safety products, including a range of beta shielding devices. The laboratory equipment section includes acrylic and stainless-steel labware, cryogenic accessories, and liquid handling equipment.

Nalge's free 1989 Nalgene **labware catalogue** contains details on over 450 Nalgene products (*Reader Service No. 126*). The 144-page catalogue lists the full specifications of each product, plus care and use instructions and expanded data on chemical resistance and physical properties. Products appearing for the first time in the catalogue include a repeating pipettor, a floating microtube rack, and six new sizes of Teflon PFA bottles. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.